THE QUARTERLY REVIEW *of* BIOLOGY

VOLUME I
1926

Published by
THE WILLIAMS & WILKINS COMPANY
BALTIMORE
U. S. A.

THE QUARTERLY REVIEW OF BIOLOGY

RAYMOND PEARL, *Editor*
The Johns Hopkins University

R. W. HEGNER, *Associate Editor*
The Johns Hopkins University

ADVISORY BOARD

CONTENTS

No. 1, January, 1926

No. 2, April, 1926

No. 3, July, 1926

No. 4, October, 1926

VOL. I, NO. 1 JANUARY, 1926

THE QUARTERLY REVIEW *of* BIOLOGY

FOREWORD

IT IS reported that there exist in the world today approximately 25,000 reputable scientific journals, devoted in whole or part to the publication of the results of research. In the face of such an overwhelming statistic it is entirely appropriate to raise the question: Why start another? As THE QUARTERLY REVIEW OF BIOLOGY stands, at the moment, in the position of the latest addition to the already large population of scientific journals, there is an obligation to make some statement as to its *raison d'être*. This obligation may fairly be judged a moral one, because, like all forms of population growth, that of scientific journalism shows definitely a tendency to approach a state of troublesome saturation. It has already become a physical impossibility for any person, professional or lay, to read carefully and critically in its original form the whole of the published output of new results of research work in any general field of science, such as chemistry, physics, biology, etc., even if he attempted to do nothing else. The same thing is indeed true of all the special fields of science except the narrowest.

At the same time it is apparent to the philosophically minded person that one of the most significant trends of science in the twentieth century is its integrative tendency. No science is any longer regarded, by even the narrowest specialist, as sufficient to itself. Biology cannot advance completely separated from physics, chemistry, and mathematics. The biologist who expects to deal with his problems in any degree of penetration, has got to know a good deal of these other subjects, and this knowledge in the long run has got to be something more than the elementary text book sort. Similarly, how far can an astronomer expect to get today without being a good deal of a physicist, or a physicist without being a mathematician, or a physiologist without being a chemist? This tendency may be interpreted to make a somewhat belated recognition on the part of Science that Nature is itself a highly integrated piece of business, of almost infinite complexity. When one starts to take it apart and put it together again, to the end of seeing how it works, he will be simply showing ordinary common sense if he has in his working tool kit as many different kinds

of wrenches, chisels, hammers, yard-sticks, etc., as possible.

These two tendencies, the ever-increasing output of highly technical results, on the one hand, and the ever-increasing necessity for the successful researcher to know a good deal about other fields of science than his own specialty, on the other hand, are patently antagonistic. Something has got to be done to bring about an effective working compromise between them. One highly important step in this direction is the development of abstract journals. This development has reached a high pinnacle of success in this country in the field of chemistry, where, in *Chemical Abstracts*, we have a model journal of its type. There is every reason to hope and to believe that *Biological Abstracts*, under the aegis of the National Research Council, will be equally successful in its field.

While abstract journals are a vital necessity to the progress of science, and are to be in every way encouraged and still further developed, they do not wholly solve the problem. There are two reasons why they do not. In the first place they tend themselves to become so large, in spite of every conceivable mechanical condensation in the way of abbreviation and the like, that no one can read them *in toto*. In the second place, in spite of the cleverest classification, arrangement, and indexing of the material, abstract journals necessarily lack in significant degree the qualities of coördination and integration. They present to the enquiring reader a vast assemblage of trees but no adequate picture of the forest. But it is precisely picture maps of the various developing forests of science that we most need if we are not to go wholly mad, and see our civilization perish because all the persons capable, by genetic endowment, of leading it, are confined in asylums as the result of a hopeless confusion of ideas.

The Quarterly Review of Biology, with becoming modesty but nevertheless courageous mien, steps forward to do its bit towards postponing or perhaps altogether averting such a devastating catastrophe. It will offer to the reader authoritative and comprehensive reviews of the present state of knowledge in the different fields of general biology. The articles will be written by specialists, who alone are capable of giving accurate, balanced and critical summaries of the present position in various lines of inquiry. The first function of The Quarterly Review of Biology will be to help the man of science, whether biologist, chemist, astronomer, or devotee of any other *Fach*, to keep soundly oriented as to the general progress of biology.

It is hoped that it may also serve usefully another group of readers. There is a marked recrudescence just now of the age-old warfare between religion and science. Some of our scientific *confrères* appear either to have forgotten, or never to have grasped, the significance of the length and bitterness of the struggle which finally freed the human mind from the shackles imposed and maintained through centuries by organized religion. They go about plaintively bleating that there *is* no conflict between science and religion, to the great joy of the shrewder Fundamentalists, who perceive at once the enormous aid which such views will be to them in their work of forging again the fetters from which Huxley finally freed human-kind. The plain fact, of course, is that there can never be any real compromise between science and mysticism, between the real and the supernatural, between light and darkness, between the demonstrable, verifiable truth which

makes us free and ancient superstitions grounded in phallic folk-lore.

The experience of centuries indicates that there is only one way effectively to meet the menace of Fundamentalism, which in a real sense threatens the continuance of those elements in our civilization which every intelligent man holds dear. It is by the diffusion of scientific knowledge. Man's emancipation from the domination of priesthoods of one brand or another has been in precise proportion to the depth and breadth of the understanding of nature which science has given him. We hope that THE QUARTERLY REVIEW OF BIOLOGY may help in the diffusion of a sound knowledge about biological matters among intelligent men and women who are not professionally scientific workers. No attempt will be made to attain this end by the method of writing down to the supposed intellectual level of a lay audience. Popular scientific writing which takes as its target a mental age of about ten, is, we believe, quite as nauseating to intelligent lay readers as it is to men of science. But we think that there exists in this country a fairly numerous body of cultivated men and women who are genuinely interested in knowing about the progress which biology is making, and quite as capable of understanding a clearly written and logically ordered presentation of it, as is the average physicist or chemist, or indeed the specialist in biology itself who cultivates a field remote from the particular one under discussion. To this group also we offer THE QUARTERLY REVIEW OF BIOLOGY.

RAYMOND PEARL.

THE BIOLOGY OF THE MAMMALIAN TESTIS AND SCROTUM

By CARL R. MOORE

Hull Zoölogical Laboratory, University of Chicago

IN DEALING with some phases of the biology of the mammalian testis a limited discussion may follow two major lines of interest: (1) a consideration of certain influences on the germinal portion of the organ—spermatogenesis—and (2) a brief discussion of the testis as an organ of internal secretion. The products of these two testicular functions have at times been referred to respectively as the external and internal secretions; the study of spermatogenesis is the older and perhaps better understood of the two, but recent study has largely concerned itself with internal secretions, almost to the exclusion of the primary function of germ cell production. We shall consider first some of the general conditions that influence the production of germ cells, particularly the functional significance of the scrotum; secondly, the internal secretory function will receive our attention.

I. GENERAL CONSIDERATIONS

The testes of most mammals are located outside the body cavity in a pouch, or scrotum, embryologically a derivative of the peritoneal cavity. A duct, the ductus (vas) deferens leads away from the epididymis to convey spermatozoa to the exterior. Embryologically, the testes arise from the genital ridge of the urinogenital fold high up in the body cavity (2) (6), and are fundamentally, therefore, organs of the abdomen; they remain so in all vertebrate classes except mammalia. In this group a new condition has made its appearance in that the testes, though arising near the kidneys, become progressively displaced posteriorly and in the majority come finally to be located in the scrotum (before birth in man). In some mammals (rodents, etc.) the pathway of descent remains open throughout life, but in man and most mammals the inguinal canals become closed.

The factors involved in the backward regression of the testis, both phylogenetically and ontogenetically, are not entirely clear. Embryologically a cordlike ligament, the gubernaculum (Hunter), exists between the developing testis and the anterior abdominal wall, and it is supposed by some anatomists (42) that muscular fibers of the gubernaculum exert a continuous pull on the testes toward the anterior abdominal wall while others (6) believe that the principle of unequal growth of the body in comparison with the fibrous cord will explain the backward regression of the testis in ontogenetic development.

Too little attention, perhaps, has been given to the exceptional formation of the scrotum in mammals. Its significance or function has been lightly touched until recently and a few enlightening facts may give us a new conception of this relatively recently acquired structure. Herbert Spencer (173) in discussing the general principles of biology speaks of the

peculiar condition found in mammals in the following terms:

> But now let it be confessed that all phenomena of organic evolution must fall within the lines above indicated, there remain many unsolved problems. Take as an instance the descent of the testes in mammalia. Neither direct or indirect equilibration accounts for this. We cannot consider it an adaptive change, since there seems no way in which the production of sperm cells, internally carried on in the bird, is made external by adjustment to the changed requirements of mammalian life. Nor can we ascribe it to survival of the fittest; for it is incredible that any mammal was ever advantaged in the struggle for life by this changed position of these organs. Contrariwise the removal of them from a place of safety to a place of danger would seem to be negatived by natural selection. Nor can we regard the transposition as a concomitant of re-equilibration since it can hardly be due to some change in the general physiological balance (page 575).

The monotremes, most edentates, sirenians, cetaceans, some proboscidians, and seals among carnivores possess complete or nearly complete abdominal testes (209). In many edentates and rodents the testes may be abdominal for the majority of the year but temporarily descend during the breeding period; this is essentially true in the Chiroptera. The majority of ungulates, carnivores, and all primates have a scrotum, and closure of the inguinal canals separates the testes from the abdomen. In seasonal breeding animals the testes are usually abdominal until the approach of rut when the testes become enlarged and reside in the scrotum.

In general it appears that an evolution of the scrotal conditon has occurred and Wiederscheim (209) states "originally, the descent of the testes did not occur until sexual maturity in all cases, but in many mammals (e.g., marsupials, ungulates, carnivores, primates) the process has gradually become shifted backward ontogenetically to earlier periods, so that the formation of the scrotum takes place independently in the embryo in the form of the external genital folds. . . . ." Cunningham (43) discussing its occurrence, evolution and significance remarks "Various causes have been suggested for the formation of the scrotum, but no one has even been able to suggest a use for it. It has always been quite impossible to bring it within the scope of the theory of natural selection. The evolution of it can only be explained either on the theory of mutation or some Lamarckian hypothesis" (page 147). In his further remarks Cunningham removes it from the possibility of having arisen through mutation and supports the Lamarckian hypothesis put forward by Woodland (212) in 1903. Woodland states

> I hold that, in the majority of the mammalia, the testis attachment has throughout the history of the race been constantly subjected to severe strains consequent on the character and conditions of mammalian locomotion, and that on account of the resulting disruption or distention of the mesorchium, the testis has migrated in a posterio-ventral line (i.e., in an opposite direction to the forward and upward accelerations imparted) coming to lie at the terminal periphery of the body wall and forming the scrotal protrusion. Just as when a man runs, a weight in his coat-pocket will periodically drag and ultimately wear a hole in the lining by constant distention, so the testis of mammals has responded to like forces resulting in descent (page 325).

Speculations on the mechanistic procedures and forces employed, are perhaps less fruitful than have been some of the more recent enquiries into its possible function, since the former conception is excluded from, but the latter aspect subject to, experimental analyses. Can we therefore offer any approach to a functional analysis of this structure which Cunningham stated a few years ago had never been attempted?

Recent work in this laboratory (121, 122, 123, 125, 128, 131) we believe has proved that in the higher mammals *the scrotum is a structure whose function is the*

maintenance of proper temperatures for the testes to carry on the differentiation of germ cells; it is an essential thermoregulator for the testes and higher mammals are incapable of producing spermatozoa without such a regulatory control provided by the scrotum. For descriptive purposes a bit of general histology is necessary.

Figure 1 is a portion of a cross section through a rabbit testis; though used for an experiment it shows normal seminiferous tubules in cross section. The ***germinal epithelium*** composed of spermatogonia, spermatocytes, spermatids and spermatozoa represents a typical condition for all mammals; spermatozoa may be seen projecting into the lumen of the tubules. Between the seminiferous tubules can be seen a small amount of connective tissue among which are a few larger cells; these are the ***interstitial cells*** or cells of Leydig (86) considered by many workers as the source of the internal secretion of the testis. These will be considered more in detail in section IX.

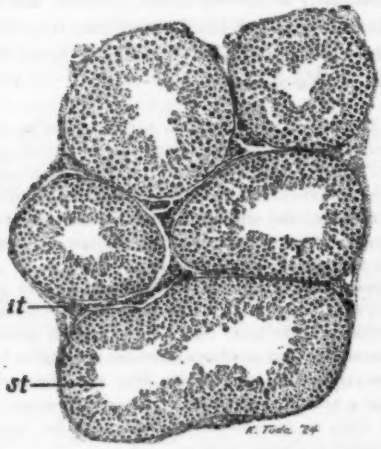


FIG. 1. SECTION THROUGH CENTER OF RABBIT TESTIS SIX MONTHS AFTER VASOLIGATION (LEFT TESTIS OF FIGURE 6, *B*)

st, normal seminiferous tubules; *it*, interstitial tissue

II. CRYPTORCHIDISM

Sometimes the testes of man and higher mammals are not to be found in their normal scrotal position; having failed to descend they may be located in the abdomen or in the inguinal canal and are hidden or cryptorchid (for structural condition see figs. 4 and 5). The condition has long been known but the oldest account to which I have made reference is that of John Hunter, that very keen anatomist of the 18th century. Hunter (75) in a lecture given in 1756 discussed the site of origin of the testis of mammals in the abdomen, and the gradual descent of these into the scrotum in the human foetus at about the eighth month of foetal life. He realized the occasional imperfection due to failure of descent. "Sometimes in the human body, and in many other animals, and very often in sheep, the testes do not descend from the cavity of the abdomen till late in life, or never at all." He suspected that the cause for this failure to descend "Originates in the testicles themselves." Their condition was also partly appreciated for "When one or both testicles remain through life in the belly, I believe that they are exceedingly imperfect, and probably incapable of performing their natural functions, and that this imperfection prevents the disposition for descent from taking place" (page 56).

Goubaux and Follin (57) studying testes retained in the abdomen in man, sheep, horse, pig and dog stated that usually the abdominal testis was both less voluminous and lighter in weight than the scrotal testis on the opposite side. These writers stated that abdominal testes did not produce spermatozoa and that retention of both testes rendered an animal sterile. Godard (54) determined that seminal fluids from men with both testes retained in the abdomen did not

contain spermatozoa, nor did cadaver cryptorchid testes show spermatozoa. Monod and Arthaud (111) studying the abdominal testes removed from man were of the opinion that younger individuals retained a diminished capacity of producing spermatozoa. Individuals of later life however were rendered sterile from traumatic influences and resulting sclerosis of the organs. In the end all ectopic testes were, according to them, deprived of their spermatozoon producing capacities. Griffiths (59) pointed out that testes of the dog and man retained in the abdomen or in the inguinal canal were degenerate so far as sperm production is concerned. In those testes located intermediate between the abdomen and scrotum the seminiferous tubules were devoid of germinal epithelium. He emphasized the necessity for the testis to completely descend into the bottom of the scrotum before they can carry on their gametogenetic function. Regaud and Policard (149) called attention to the lack of germinal tissue in the undescended testis of the pig and mention particularly the considerable apparent increase in interstitial tissue.

Some of the most exhaustive work on undescended testes in the human is that of Felizet and Branca (45, 46). These investigators studied a series of abdominal testes from six year old boys to adults and point out that seminiferous tubules are the characteristically affected portions of the gland, the epididymis remaining normal in structure. In ectopic testes removed from a preadolescent individual the single layered undifferentiated germinal epithelium differed but little from that of similar aged normal testes. As the period for the differentiation of spermatogonia, spermatocytes and spermatids approaches, degeneration follows and the epithelium never reaches the completely differentiated stage. Whereas later writers, and some earlier ones, believed cryptorchid tests at puberty did reach and maintain spermatozoon differentiation for a short time, Felizet and Branca were unable to find a single case among the fifty-one studied where this appeared true. Of this number they report sixteen cases with spermatogonia present; twelve cases with differentiation to the spermatocyte stage, and only two cases, of the fifty-one, showing spermatids: no cases with spermatozoa present were found. The interstitial tissue was usually more abundant in normal testes but the number of Leydig cells varied considerably.

Bouin and Ancel (22) studied ectopic testes of the boar and dog and noted in all cases that the seminiferous tubules were smaller in caliber, and contained only the cells of Sertoli; the interstitial cells were usually abundant. Corner (40) studied histologically sixteen cases of ectopic testes removed from man but did not find evidences of spermatogenesis in any case. Whitehead (205) similarly found that an undescended testis of the horse was degenerate so far as the germinal tissue is concerned. Hanes (65) studied fifty-six undescended testes from the pig, seven from the sheep, and six from man. He states that in no case was spermatogenesis in progress and the tubules contained only Sertoli cells; the intertubular spaces were well filled with masses of Leydig cells. Bland-Sutton (20) studied histologically the ectopic testes removed in the course of twenty-five years surgical work (number not given) and mentioned that only one contained spermatozoa. He believes that ectopic testes develop to their normal differentiated condition at puberty and retain the capacity to produce germ cells for a year or two. His opinion however, receives but little support from the studies of most investigators but it may be men-

tioned here that Moore (122) studying experimental displacement of the guinea pig testis found that such an organ retained in the inguinal canal with one pole projecting into the upper portion of the scrotum might produce normal germ cells in one part and not in another. It is possible that the single case bearing spermatozoa reported by Bland-Sutton was a low inguinal canal retention instead of an abdominal one. Goddard (55) studied five cases of the retained testis removed from men 19 to 25 years and in no case was there any indication of spermatogenesis, though the interstitial cells were well preserved and apparently present in an overabundance.

What to do with an undescended testis is a problem that ever confronts the surgeon, and because the fundamental conditions of the gland and its potentialities have not been understood there have been many opinions voiced and different procedures employed. Even in Hunter's period (1756), and purely on philosophical grounds, he argued that nature should be assisted in all ways possible to bring the testis into its normal locality in the scrotum. Later the idea that an ectopic testis is decidedly prone to develop neoplasms and metastasize became more or less prevalent and tended to encourage removal of such organs. The knowledge that an ectopic testis can still exert its internal secretory function, however, has led many to remove the organ from the inguinal canal, in order to cure or lessen the tendency to hernial formation, and allow it to remain in the abdomen. Thus Corner in 1904 reviews the general question of the value of an incompletely descended organ and recommends replacing it in the abdomen. He believes it useless to attempt a scrotal return and considers the testis of no use except as an organ to produce sex hormones. Bland-Sutton likewise, believing the ectopic organ useless, exercises but little care in its preservation. He and others, believe the organ was retained in the abdomen because it was intrinsically defective. Keyes and MacKenzie (78) on the other hand advise an operation to place the undescended testis in its normal scrotal position before the stage of puberty, thinking, evidently, that a mere possibility remained for it to develop into a functional condition. Bevan (18) describes a technic for restoring the ectopic testis to its position in the scrotum and advises this operation.

The advocacy of the operation on the human individual, however, is from the very nature of the case difficult to determine. Furthermore since the real fundamental condition of, or the potentialities of, an abdominal testis have not been understood a rational basis for procedures has not been clearly indicated. The question of why a testis is found in the condition of degeneration in over ninety-nine per cent of all reported cases should be settled. Of necessity this involves animal experimentation and it is from this field that many new facts have been derived.

Up to about the year 1890 three different conceptions had been advanced to explain the condition of naturally occurring cryptorchid testes: (a) It was believed by some that the abnormal condition was due to an imperfect embryological development and that its structure rested primarily upon the faulty formation of the organ. (b) Some authors held that early development was normal, but differentiation of the germinal products failed to pass beyond an embryonic stage of the germinal epithelium. (c) Others believed that the testes acquired the capacity to produce spermatozoa, retaining same for a year or so, but on account of the abnormal position occupied regression takes place and leaves

the organ deprived of its germinal tissue.

The English anatomist Joseph Griffiths (58) is to my knowledge the first individual to attempt a solution of the problem by experiment. In 1893, working with the dog, he demonstrated that a testicle of a puppy that had descended normally into the scrotum would never produce spermatozoa if it was returned to the abdomen by an operation, despite the fact that its vas deferens, blood supply and nerve connections were entirely normal. Such a testis developed but little farther than the embryonic stage of differentiation; the opposite scrotal testis differentiated normally. The testis of an adult dog, actively producing spermatozoa, would within a few months after having been replaced within the abdomen, show a condition similar in all respects to abdominal testes; all the germinal tissue vanished and only the single layer of Sertoli cells remained. He proved therefore that the structural condition of an ectopic testis was not due to any inherent defect within the organ but was a function of its abnormal environment. Some influence from its location was responsible for its condition and he speculates at some length on the situation. "What, then, can be the reason that in some, a limited number of animals, the scrotal position is essential to structure and function? . . . . can it be that the very turbulence of this region is requisite for its perfect development and the maintenance of its functions in certain animals? . . . . So far as I am aware, no satisfactory answer has ever been offered to this most interesting question" (pp. 496–497). Though not able to determine the active influences brought to bear upon the testis in its abnormal location Griffiths' tendency was distinctly directed to the scrotal relationships as a solution. He emphasized the necessity of the testis descending completely to the bottom of the scrotum before it could exercise its normal functions, and furthermore compared the condition of the testis of seasonal hibernating mammals with the abdominally retained testis of scrotal animals.

The Danish investigator Knud Sand (160) utilized the method of Griffiths in replacing the normally descended and actively functioning adult testes of rats and guinea pigs into the abdomen. His study, however, did not advance our knowledge of the problem of the degenerate testis when it is located in a strange environment, for his chief interest lay in the behavior of the interstitial tissue which he believes becomes greatly increased. He confirmed Griffiths' statements that normally functioning adult testes when replaced in the abdomen are converted into typical ectopic testes within a few months; the germinal epithelium that was actively producing germ cells at the time of elevation had all disappeared within the course of three months with only the single layer of Sertoli cells remaining.

This problem along with many others was being studied in this laboratory in 1921 (Moore 118). An attempt was being made to determine the real causes of the degeneration of the testis when placed in the abdomen. By experiment such possibilities had been eliminated as infection, physical connections with some part of the scrotum, abnormal pressures, and disturbance of blood or nerve supply and it was stated that a differential body temperature was probably the causal factor in degeneration; experiments testing this hypothesis, though under way, were incomplete.

Crew (41) purely on hypothetical grounds suggested that the cause of the lack of spermatozoa in the imperfectly

descended testis might be due to a higher temperature in the abdomen than in the scrotum, considering that it was this higher temperature that might be responsible for the non-appearance of an active epithelium. He thus was the first with a suggestion that has proven in our laboratory to be the causal factor in the structural condition of a testis either never having descended from the abdomen, or having descended and accomplished its complete differentiation reverts to the typical ectopic type when by experiment it is returned to the abdomen.

Moore (122) reporting more in detail on the observations made with experimentally produced cryptorchid testes found that a normal adult functioning testis of the guinea pig shows decided degeneration of the germinal epithelium within six days after being elevated from the scrotum to the abdomen (see fig. 2). This degeneration is noticeable from the fact that the seminiferous tubules are disorganized, and many loosened cells occupy the lumen filling the latter with discrete cells some of which are normal and others fragmented; a considerable number of tubules contain only degenerate cells and a mass of debris. Somewhat later than the sixth day after elevation the tubules may have sloughed the major portion of the germinal epithelium and the remaining cells may have coalesced into multinucleate protoplasmic masses or "Giant Cells" (see fig. 3). By twenty days practically all the former active germinal epithelium was absent; Sertoli cells and a few spermatogonia remained. The interstitial cell mass appeared progressively more conspicuous, but the significance of these will be dealt with more in detail in section IX.

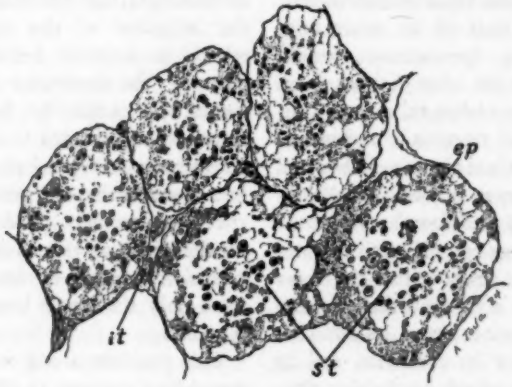


FIG. 2. SEMINIFEROUS TUBULES OF ADULT GUINEA-PIG TESTIS SHOWING DEGENERATION SEVEN DAYS AFTER ELEVATION TO ABDOMEN

ep, degenerate epithelial wall; *it*, interstitial tissue; *st*, seminiferous tubules with degenerating cells in lumen

So long as the testis remained in the abdomen it continued degenerate; followed for longer than a year the organ as a whole progressively diminishes in size and the seminiferous tubules shrink to one-fourth or one-fifth their normal diameter. If, however, the testis in a partial descent into the scrotum was held within the inguinal canal with its lowermost end projecting into the upper portion of the scrotum there might be found one portion in which the tubules could still carry on their gametogenetic function, though ap-

parently in a diminished capacity, while another portion located more closely to the interior of the abdomen was entirely degenerate.

It was desirable to learn more of the potentialities, of such a degenerate testis, and to determine if it retained the capability of regenerating into a normal functioning gland. Moore determined that recovery did occur if the testis was replaced in the scrotum. Twenty-four days

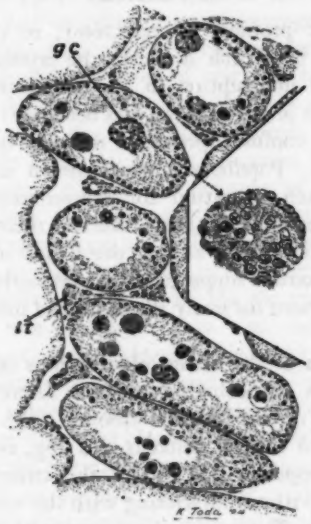


FIG. 3. TUBULES OF ADULT GUINEA-PIG TESTIS CONFINED TO ABDOMEN THIRTEEN DAYS

gc, multinucleate giant cells formed from coalescing germinal cells

abdominal retention reduced seminiferous tubules to a single layer of cells among which were a few spermatogonia; return of such testes to the scrotum led, within three months, to testes with normal tubules and quantities of spermatozoa. Thus it is seen that the testis must reside in the scrotum to be able to carry on its function of gametogenesis but the limits of degeneration with recovery still possible have not yet been determined.

It would appear therefore that the surgeon should take especial care to see that ectopic testes are restored to their normal environment. Their degenerate condition, while it may be so, is not necessarily due to faulty embryological formation. Davis (44) has recently enumerated as causes for lack of scrotal descent (1) defects in the mesorchium, (2) paralysis, absence, or faulty insertion of the gubernaculum, (3) narrowness of the vaginal

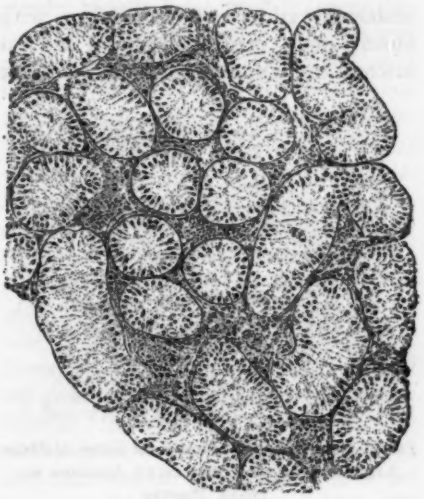


FIG. 4. SEMINIFEROUS TUBULES FROM UNDESCENDED TESTIS OF AN ADULT DOG

process, or large size of the testicle, (4) shortness of the spermatic cord, (5) rudimentary or obliterated scrotum, and (6) premature obliteration of the inguinal canal. It seems probable that placing the testis in the scrotum by operation would lead to its normal development in such cases. Operation before puberty certainly carries many strong chances that a child with doubly undescended testes may be given the chance of acquiring his normal germ cell production capacity by a small operation, whereas without it there is no

chance of his acquiring this function. How long after puberty a testis returned by operation to the scrotum can regenerate into a normal organ has not yet been determined.

In addition to studying experimental cryptorchidism in the guinea pig we have performed similar experiments on rats, rabbits and sheep. Thus Moore and Oslund (131) determined that a normal breeding ram testis removed from the scrotum and replaced in the abdomen surrounded by all its natural coverings, with blood supply, nerve connections and vas deferens in a normal condition, would be

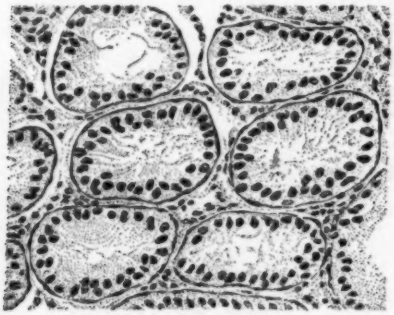


FIG. 5. SEMINIFEROUS TUBULES FROM TESTIS OF ADULT RAM REMOVED FROM SCROTUM TO ABDOMEN FOR THREE MONTHS

rapidly converted into a degenerate testis with a single layer of Sertoli cells in the seminiferous tubules (see figs. 4 and 5) Moore and Quick (129) obtained similar results in the rabbit. In the rat (Moore, details unpublished) degeneration follows in the testis, elevated and confined in the abdomen, but the degree of degeneration is by no means so pronounced as that in the guinea pig, sheep and rabbit. A rat testis confined to the abdomen for a period of six months is considerably degenerate and no tubules of a normal character can be found, but they are more nearly normal than a guinea pig testis similarly retained for fifteen days. It appears that the rat testis can tolerate greater ranges of temperature than can the guinea pig testis. This may be accounted for by supposing that the rat testis is not so dependent upon a finely graded regulation of its environment as is the testis of the guinea pig and consequently represents a lower grade of differentiation as regards the scrotum (see section V).

III. VASECTOMY

The problem of vasectomy, or vasotomy, by which is meant the production of an interruption in the course of the ductus or vas deferens, has been one of the most confused problems relating to the testis. Popularized it is known as the Steinach operation for rejuvenation; it has served at the same time as a theme for popular fiction and in the hands of the surgeon as a supposed efficacious method of treatment for many ills of human individuals.

One of the earlier observations on the effects of occluding the vas deferens is that of Sir Astley Cooper (39). He ligated one vas deferens in a dog, and on the opposite side ligated the artery and vein without interfering with the vas deferens. The testis whose artery and vein were ligated "gangrened and sloughed." Operated in 1823 the dog was kept until killed in 1829. During six years it was noted in coitus two separate times but no issue followed. Observed after killing, the testis with its epididymis was notably increased in size and the latter, with the short stub of appended vas deferens, was gorged with spermatozoa. Occlusion of the duct had been complete and the two ends of the severed duct were separate. This conclusive experiment showed without doubt that merely closing the outlet duct of the testis had no effect upon the germinal portion of the organ;

spermatogenesis continued for six years after operation and the epididymis became much enlarged to accommodate the products of germinal cell activity.

Gosselin (56) some years later dissected human cadavers and noted cases in which the vas deferens was entirely occluded, and had been undoubtedly for years, but the enlarged epididymis contained quantities of spermatozoa. Brissaud (19) and Griffiths (61) with rabbits and dogs respectively found that occlusion of the outlet duct had no influence on spermatogenesis. Griffiths (62) a year later, however, determined that testes of the dog rapidly lost the germinal portion if blood vessels passing to the testis were injured. Simmonds (170) in human autopsies found occlusion of the vas deferens of years standing without any injury to the generative portion. Neither testicular degeneration nor loss of the germinal epithelium had occurred and the epididymis was enlarged from the immense amount of spermatozoa carried to it.

Thus in man, dogs and rabbits it was well established by incontrovertible data before 1900 that blocking the outlet duct from the testis had no effect in causing the testis to become aspermatic. Gametogenesis continued for years and quantities of spermatozoa were to be found in the epididymis and the proximal portion of the vas deferens.

About 1903 when results on experimental studies of the testis became more numerous contrary results began to appear. Richon and Jeandelize (156) stated that rabbit testes, whose vas deferens had been experimentally occluded contained no spermatozoa, and that the seminiferous tubules were degenerate. The extensive study of sex glands conducted by Bouin and Ancel, however, was the chief factor in changing the tide of opinion relating to the effect of vas deferens occlusion. Summarizing many of their different studies Bouin and Ancel declare that closing the outlet from the testis, by ligation and resection of a portion of the ductus deferens, invariably leads to degeneration of the germinal tissues of the testis. They worked with guinea pigs, dogs and rabbits, young and adult. According to their account ligation of the outlet duct before the stage of puberty does not interfere with the attainment of complete germ cell differentiation, but after this attainment the seminiferous tubules rapidly loose their lining epithelium and become converted into a testis entirely similar to the cryptorchid testes found in nature. They dismiss the contrary findings of the earlier writers by assuming an insufficient length of time after operation before the animals were killed. It should be remembered that Cooper's dog was not killed until six years after the operation for closure of the tube, and pathological occlusion in man had been present for years without testis degeneration. The question at issue is not whether Bouin and Ancel obtained degeneration of the germinal epithelium after vas deferens ligation, for undoubtedly they did; the real question is whether the degeneration they saw was due to the occlusion of the duct or whether factors not necessarily related to this operation are the real causes.

Shattock and Seligman (169) performed double vasotomy on Herdwick rams, but despite the fact of double, complete vas deferens occlusion for eighteen months the testes were normal in size and spermatogenesis continued; the epididymis was much larger than normal due to the retention of the products of spermatogenesis and consequent distension of the epididymis. They note that testes may be abnormal if the blood vessels had been included in the ligature of the vas deferens.

Wallace (198) reviews the results of vas deferens ligation in man. Sometime before this period it was an accepted surgical practice, due to the suggestion of earlier workers, to ligate the sperm duct as a method of alleviating prostatic hypertrophy. Favorable results had been reported in a great number of cases but Wallace remarks of these "When it is remembered how often cases of prostatic enlargement are greatly improved by rest and treatment of the accompanying cystitis, it is an open question whether the results above set forth were not to a considerable measure due to such treatment." From the literature on sex it would appear that the implications of such a statement are too infrequently considered in clinical work. Wallace performed vasectomy on the cat and dog and found that the testes in such cases were perfectly normal many months later than Bouin and Ancel would imply was a sufficient time to expect degeneration. Wallace concludes that single or double vasectomy has no effect upon the gametogenetic function of the testis; it continued to produce spermatozoa months and years after total occlusion of both vas deferentia.

Kuntz (80), on the dog, stated that thirty days after unilateral vasectomy the testis of the same side showed degeneration but also on the unoperated side as well. He conceived some influence operating through the nervous system that caused degeneration on the opposite side. Later experiments (81) on both the dog and rabbit were reported in which practically all animals showed testis degeneration as the result of vas ligation and resection. In control animals, however, Kuntz noted that the testes were likewise aspermatic and recognized that confinement and care of the animals was such that the unoperated tests were degenerate to about the same extent as those of experimental animals. He then withdrew his former suggestion that sympathetic nervous influences set up by one degenerating testis caused the degeneration of the opposite one, but he allowed the idea to stand that within a month after operation the degenerate testis shows the influence from ligation of the vas deferens. Certainly no great weight can be attached to such conclusions when admittedly control animals do not show normal testes.

The greatest impetus to the conception of testis degeneration following vas deferens occlusion since Bouin and Ancel is the striking and apparently conclusive work of Steinach (1910–1920) culminating in his suggestive work on rejuvenation (183). Reviewed more in detail in a later section it may be mentioned here that the conception of the origin of sex hormones, from interstitial cells, and the properties of these hormones in producing youthful qualities in animals are the basis for his now celebrated rejuvenation hypothesis. By ligation of the vas deferens in rats Steinach reported degeneration of the germinal epithelium accompanied by an hypertrophy of the interstitial cells—the hormone producing tissue; secondarily this stimulating hormone causes renewed germ cell production. Thus he believes that an animal so old as to have lost its germ cell production capacity is reinvigorated by a unilateral vas deferens ligation, and that the opposite testis following the stimulation begins to produce germ cells again and the animal returns to a functional condition. Steinach believes that ligation between the epididymis and the testis proper hastens the degeneration, and hypertrophy of the interstitial tissue. The fallacy here again depends upon operative manipulation of the organs in question, and an absolute disregard of the very many perfectly conclusive experiments showing the testis to be unaffected by

merely binding or severing the vas deferens. This will be further emphasized below.

Tiedje (190) follows Steinach in the belief of first a degeneration of the germinal epithelium then a regeneration after vasectomy. It should be pointed out, however, that neither Steinach nor Tiedje give substantial evidence to support their contentions. Wheelon (201), on dogs nine months after vas deferens occlusion, found that the testis contained all elements of the germinal epithelium. He believes either that all the tissue did not degenerate or that there might possibly have been a regeneration.

Sand (159) (161) (163) has studied the effects of vasectomy on the rat, guinea pig and dog, and has performed the operation in clinical practice, reporting eighteen cases of such operations on the human individual. In the latter, however, Sand follows Steinach's procedure and ligates between the epididymis and testis instead of merely occluding the duct. By this method he believes that the process of destruction of the germinal tissues is hastened. Sand (159) reported observations on vasectomy in rabbits, guinea pigs, and rats, summarized in tables in his paper; out of fifteen operated cases, some of which were observed for longer than a year, two cases only showed degeneration. Despite the fact that thirteen of his own operated cases failed to show degeneration, as against two that did give such evidence, he yet states a confirmation of the work of Bouin and Ancel that vas deferens ligation causes degeneration of the germinal epithelium and proceeds to employ the operation on man. Brack (27) however has shown that congenital absence of the vas deferens in man is without effect on spermatogenesis.

In this country Benjamin (12) has been perhaps the chief advocate of the Steinach operation as applied to the human individual. In such cases histological studies are lacking but accepting the Steinach idea as proper and due to follow, Benjamin restricts himself to the clinical effects manifest in the patient. Clinical results will be granted by most individuals as being considerably conditioned by forced rest, good care, mental attitude, suggestion, and many other factors. And in view of the lack of proof of organic changes following this operation certainly one's credulity is stressed to the uttermost when it is maintained that the clinical results of this operation are alleviation of arthritis; lowering of blood pressure; reduction of prostrate (long ago definitely proven to be inefficacious); improvement of eyesight, and sclerosis of the inner ear; elimination of mental depression; improvement of sex impulse; and general restoration of spirits. We are led to believe that vasoligation first improves thyroid activity, and outside of this it influences "those glands that are most prominent or delinquent in the patients constitution."

The enormous literature on this subject (some only, being here given) is seen to abound in positive and negative assertions without any attempt to find the fundamental differences in technic or conditions that would in any way make the situation clear. Could we find an acceptable explanation to account for the cases of degeneration perhaps the entire findings would take on a different aspect and order be reëstablished out of chaos. When, as did Cooper almost one hundred years ago, we find that a testis whose vas deferens was completely severed and occluded so that all materials produced by it are confined to the testis, epididymis, and the attached portion of the vas deferens and that the testis, is entirely normal except for distention of the epididymis with the seminal

products, then there appears to be no question whether ligation of the duct, of itself, produces degeneration of the germinal portion of the organ. Many things can be done to the testis that will cause it to loose its germinal epithelium in a very short time, for this organ is known to be a very labile one and to react to many unfavorable conditions by loss of its germinal tissue.

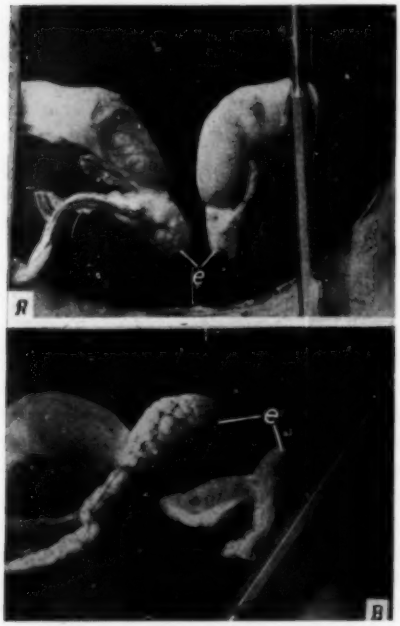


FIG. 6. PHOTOGRAPHS OF TWO ADULT RABBIT TESTES

A, testis on right, normal control: testis on left, vasoligation six months (note distended epididymis, *e*).

B, testis on right, abdominal retention six months; testis on left vasoligation six months (section of left testis in *B* shown in figure 1).

In 1919 the writer sought to produce degeneration of the testes and compensatory hypertrophy of the interstitial tissue by vas deferens ligation in order to compare these testes with testis grafts. In the rat and guinea pig, however, degeneration did not follow after operation (observations unpublished). The problem was further investigated by Oslund, then a graduate student in this department, and the first rational interpretation was given to results obtained following vasectomy (134). Correlation of results obtained with those obtained by the writer on the effects of displacement of the testis in experimental cryptorchidism (see section II) focused attention on the position of the testis following vas deferens ligation. Oslund was able to correlate all cases of degeneration following vas deferens ligation with an abnormal position of the testis where this was definitely known. Further experiments checking absolutely the position of the testis after operation showed that in every case the testis was normal if it resided in its normal scrotal position, but if it was held by adhesions in the inguinal canal or in the lower abdomen it was degenerate; a similar degenerate condition, however, followed as quickly if the vas deferens was intact but the testis abnormally situated. It follows therefore that degeneration was not due to occlusion of the vas deferens but to an abnormal position of the testis. Oslund later (135) confirmed earlier writers that vasectomy does not affect the dog testis if the dogs are not confined to close quarters, and that degenerate testes are often found in dogs confined but which have had no operation. Moore and Oslund (131) likewise confirmed earlier experiments on sheep vasectomy showing that the testes continued in active spermatogenesis months after complete occlusion of the sperm duct.

In order to test the hypothesis of an early degeneration of the epithelium followed by regeneration in vasectomy cases, Moore and Quick (129) performed the operation on a series of rabbits, studying the testes at intervals from thirteen days to

six months after operation. Spermatogenesis was normal after the operation and the retained products caused the epididymis to double or triple in size. Figure 6, *A*, shows the ligated testis of a rabbit in comparison with the normal one six months after vas deferens occlusion. One can readily see that the epididymis of the vas ligated testis is much larger than the normal. Figure 1, a section taken through the center of the testis shown in Figure 6, *B* (left), shows that the seminiferous tubules are normal and actively producing germ cells; testes removed thirteen days after operation were normal. There was no evidence that would lead one to assume an earlier degeneration of the germinal epithelium followed by a regeneration.

The enlargement of the epididymis tends to cause the testis to be elevated towards the neck of the inguinal canal and abdomen and on several occasions has been seen to result in the displacement of the testis into the abdomen. Because of the hypertrophied epididymis the testis sometimes does not immediately return to the scrotum and it should be remembered that artificial displacement of the testis into the abdomen for a very short period causes it to degenerate. In one case, we noted abdominal retention and consequent degeneration at six months. It must be emphasized, however, that this degeneration was not due to vasectomy but to abdominal retention. In other cases where purposely the testis was confined to the abdomen it was degenerate thirteen days after operation, but this is all that could be expected when the effects of abdominal retention are borne in mind. Thus it is again shown that ligation of the ductus deferens does not of itself cause the testis to degenerate, nor have we in this laboratory ever seen a case of hypertrophy of the interstitial cells following this operation. Our studies involve more than a hundred vasoligations on rats and guinea pigs, twenty on rabbits, and four on sheep, with intervals of time from two weeks to longer than a year.

It should be emphasized that degeneration of the testis and the so called interstitial hypertrophy is the very basis upon which the idea of the efficacious measures of vas ligation are based and our experiments in this laboratory have been repeated on the same animals utilized by the originators of this hypothesis. With such facts in mind how are we to account for degeneration reported by other investigators? Emphasis should be placed upon the fact that the testis is an extremely labile organ and many apparent slight influences will cause its rapid degeneration. Mere confinement of the dog often leads to loss of germ cell production, which undoubtedly is temporary.

Our point of error has been adequately demonstrated by this laboratory, namely that of displacement of the testis from its normal scrotal position with consequent degeneration from experimental cryptorchidism and not from vas deferens ligation. This is very prone to happen in animals whose testes have an open pathway from the scrotum to the abdomen, as the rat, rabbit, and guinea pig; and the majority of degenerations reported have come from this series of animals. Manipulation of the testis where trauma is involved often causes the testis to be retained partially or wholly in the abdomen. The effect of this has been known since Griffith's time but it is easily forgotten and neglected. It was so in this laboratory, and only the first hand dealing with such operations coincident with vasectomy served to impress the fact sufficiently that slight displacements were to be taken seriously. No doubt a great number of degenerations following vasectomy

are due to this factor. Operation on such animals should be done through the abdomen where the vas can be ligated close to the testis without touching the testis or with drawing it from the scrotum; operation though the scrotum itself should be rigorously avoided if uncomplicated conditions are desired. Even with a clean operation on the vas deferens, where by all means the artery of the vas deferens is to be avoided, adhesions have occurred that caused testis elevation. This condition leading to degeneration must be clearly separated from the effects of the occlusion of the vas deferens. All blood vessles running to and from the testis should be avoided. Many workers have given no heed to this complicating factor and have ligated the entire spermatic cord. It should be no surprise that degeneration follows such a procedure in the testis for organs deprived of their blood supply will undergo degeneration whether they be liver, kidney, brain or spleen.

Steinach and Sand severed the testis from the epididymis believeing that by this method degeneration occurred more surely and more rapidly. Van Wagenen (193) recently studied this method of blocking the testis outlet and notes degeneration of the testis in 106 cases in the rat. In a later paper (194) however she states that her operations were followed by hardening of the testis from congestion, and that the lack of oxygen and food was undoubtedly sufficient to cause such destruction as was obtained.

The lack of proper controls has in some cases led to misrepresentations of the effect of occluding the outlet duct. And finally many apparently trivial conditions can become complicating factors and give results that are wrong in implications unless particular attention is given to the fact that the testis is affected adversely. Insufficient attention to apparently minor details is the structure upon which has been builded the erroneous hypothesis that occlusion of the duct of the testis leads to the degeneration of its germinal epithelium. Belfield (10) has recently restated the conditions obtaining in man where the vas deferens has been totally occluded. Thus "In a large majority of subjects sperms have been found there (epididymis) even from five to seventeen years after the occluding epedidymitis occurred; in these cases, therefore, atrophy of the spermatic tubules had not occurred" (page 1242).

Founded on error, therefore, and abundantly disproved on every species of mammal where degeneration after vas ligation has been reported, it would seem that the operation as applied to man for purposes of rejuvenation should have well run its course—at least so until adequate proof of some biological principle is involved to justify its utilization. At present it may be emphatically stated there is no biological justification for the operation that has been established by experiment. The testis neither undergoes rapid degeneration solely on account of blocking the outlet from it, nor does this operation affect in any detectable manner the condition of the interstitial cells. It is now well understood that many influences can cause degeneration of the testicle, and that in the majority of such cases of degeneration the interstitial cells appear to be more abundant. In infarction of the liver or kidney, areas of degeneration of parenchyma can be seen accompanied by a proliferation of connective tissue, but as yet this method of clinical procedure has not been employed as an efficacious measure. Granting there may appear to be an interstitial cell increase, it is yet to be proven that such has any influence upon the organism. Until such a condition is proven there is no justification in accepting in principle an efficacious result

from any method that causes the testicle to degenerate. This is dealt with more in detail in a following section.

IV. TESTIS TRANSPLANTATION

Transplantation of the mamalian testis has been studied for many years and the development of our present knowledge of the best technic, of the possibilities of the reaction of the transplanted tissue, and of the efficacy of the procedure has been a fascinating topic.

The earliest attempt to transfer the testis from one organism to another with notable results dates back to 1849. In that year Berthold (17) published his notable contribution on the autoplastic and homoplastic transplantation of the bird testis. Testes placed within the coils of the intestine and removed six months later contained spermatozoa capable of movement. About forty years later Berthold's observations were confirmed for the same animal by Loge (96), Hanau (64), and Foges (48). It was found that the bird testis graft persisted in a functional condition not only in the abdomen, its normal location, but also in subcutaneous areas wherever it could obtain a vascular supply: underneath the skin the spermatogenetic function continued and motile spermatozoa were produced.

Following this earlier successful bird work, both amphibian and mammalian transplantations were studied, but in the latter, expected successes did not easily follow. Many workers were able to successfully transplant mammalian ovaries and it was realized early that these could be transplanted with much greater ease than could the testis. Ribbert (155) was unable to transplant the testis of the rabbit or dog and obtain persistence of the graft. Within a very few days after transplantation he noted that the seminiferous tubules underwent degeneration and rapidly lost their germinal epithelium; in some cases the epididymis tubules could be found in a relatively normal condition but more often all the graft was resorbed and replaced by connective tissue. Ribbert believed that it was impossible to transplant the organ successfully because of the fact that its functional products were discharged to the outside. Gobell (53) likewise failed to obtain persistence of transplanted testes of the rabbit and guinea pig. The grafts rapidly became necrotic and gave no evidence of a capacity to persist and carry on their spermatozoon producing function; the contents of the seminiferous tubules at the time of transplantation were rapidly absorbed. Foa (47) failed in his attempt to transplant the dog testis and he was firmly convinced that the testis could not be transplanted and persist. Cevolotto (35) was somewhat more succesful with the rabbit; grafts recovered forty-five days after subcutaneous transplantation consisted of a few degenerate tubules. Practically all the germinal epithelium was destroyed by a process of sloughing into the lumina of the tubules and subsequent degeneration. Castle and Phillips (33) had negative results in thirty-three attempts to transplant young rat testes into the empty scrotum of an older castrated rat.

Beginning with the work of Steinach better results were obtained in transplantation of the testes (177 to 183). In 1910 he reported the successful persistence of autoplastic testicular grafts but in all cases the seminiferous tubules were entirely abnormal in as much as only tubule outlines containing a few Sertoli cells were present instead of the normal germinal content characteristic of the organ. He states that in all testis grafts the germinal portion is lacking and that the most prominent tissue is the interstitial tissue composed largely of the cells of Leydig;

these he considers to be the source of the sex hormones (see later section).

Voronoff (196) has transplanted the testes of goats, rams, and man. Retterer (151) (152) describing these grafts recovered by Voronoff from the goat and ram mentions that the majority of the grafts are degenerate; may times there is only connective tissue, but in others well defined tubule outlines are present with at times traces of the germinal epithelium. One testis graft from each, the goat and the ram, are said to show a fairly normal epithelium within the seminiferous tubules and to contain bodies believed to be sperm heads. This was true in the goat graft (removed from the scrotum) after a period of persistence of about two weeks. It may be questioned, however, whether this graft represents possible retention of completely differentiated products at the time of transplanatation; it is conceivable, in the absence of other grafts to substantiate the idea, that this graft represents a retention of previously formed products rather than the differentiation of new products after transplantation. This short period of time leaves the implication of the findings somewhat in question. A longer persisting ram testis graft showing fairly normal seminiferous tubules containing sperm head-like bodies, is also open to question for the reason that the graft was fastened to the normal testis of the host. In removal of this graft it may be questioned whether a portion of the normal testis in situ was adherent to what was supposedly the original transplanted tissue.

The short account of Retterer was unknown to Moore (119) when he stated that for the first time mammalian testis grafts were obtained which had differentiated spermatozoa. If the implications of Retterer's account are correct then this was actually shown two years earlier. It makes little difference whether actually correct or not, the significant thing, and the point contended for by Moore, is that there exists but one locality in the mammalian body where grafts have proven capable of producing spermatozoa. This locality is the scrotum, and its significance is discussed in section V.

Sand (157, 158, 162, 164) has reported his experiences many times with transplantation of the mammalian testis, using chiefly rats and guinea pigs. His general results on histological persistence of the testis grafts differs in no material way from those described by Steinach, except that he does mention (164) that sometimes the germinal epithelium may be partially preserved and contain spermatogonia and spermatocytes.

The results of testis transplantations in this laboratory were at first similar to those described above (Moore 115, 116); grafts consisted of degenerate seminiferous tubules containing only Sertoli cells, and many times an apparent overdevelopment of interstitial cells though it was recognized that the latter did not always follow. Figures 7 and 8 are reproductions of small portions of two grafts obtained from transplantation of the testes into spayed female guinea pig. It is evident from figure 7 that seminiferous tubules lack a germinal epithelium except for the single basal row of Sertoli cells; interstitial cells appear to be developed in an overabundance. Figure 8, however, is an exceptional type and shows that active spermatogenesis has continued despite the fact that the graft resided in the spayed female for a period of nine months. The interstitial cells in comparison with figure 7 are minimal in amount and approach the condition of this tissue in the normal testis. From this the question arose why spermatozoa were never found in transplanted mammalian testes; Berthold had obtained

bird testis grafts with spermatozoa. If in mammals the earlier phases of spermatogenesis continued actively, why were later stages with actual spermatozoa never found? It can be seen (*a*, fig. 8) that the lumen of some of the tubules contained loosened cells in stages of degeneration. On closer observation it could be seen that

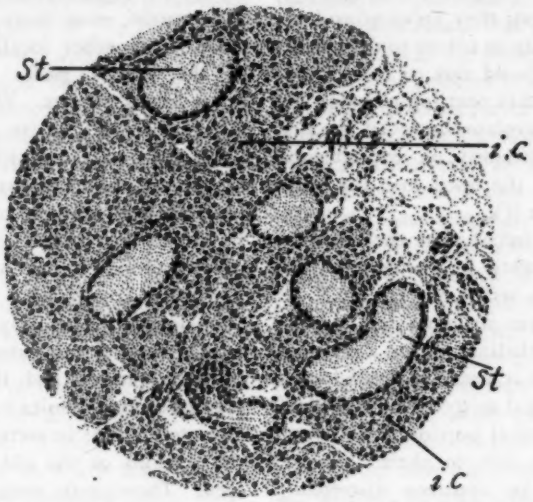


FIG. 7. GUINEA-PIG TESTIS GRAFT (PERITONEAL) SEVEN MONTHS IN SPAYED FEMALE
ic, interstitial cells; *st*, seminiferous tubules

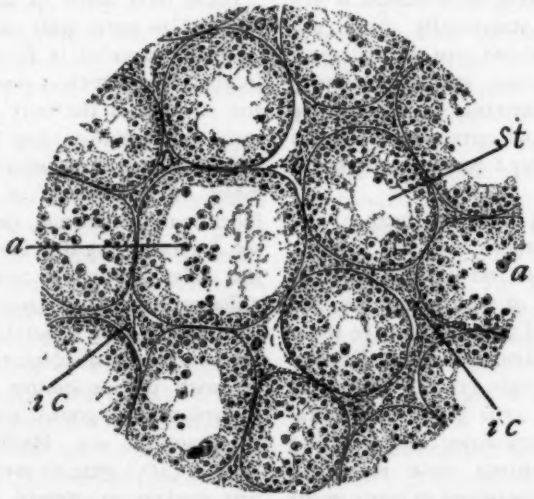


FIG. 8. GUINEA-PIG TESTIS GRAFT (SUBCUTANEOUS) NINE MONTHS IN SPAYED FEMALE
a, cells sloughed from epithelium into tubular lumen; *ic*, interstitial cells; *st*, seminiferous tubules.

these were gradually escaping from the medial edge of the epithelium into the lumen where they underwent fragmentation and solution; they appeared to have lost their capacity to adhere to the epithelial strata and build this to completion. The answer to this puzzling question did not receive its explanation until the first results were obtained on experimental displacement of the testis into the abdomen (see section II).

When it was forced upon the attention that a normal testis in a stage of active spermatogenesis will undergo rapid degeneration within six days and lose all its germinal epithelium within a period of twenty days if it is placed in the abdomen, it was only logical to account for the lack of the the germinal portion of the testis grafts as being due to their abnormal environment. An apparent discrepancy should be made clear. It has been found that a testis displaced from the scrotum into a subcutaneous environment is entirely different structurally from the opposite one displaced into the abdomen. And whereas many grafts previously described in the literature have been made intramuscularly and intraperitoneally the one shown in figure 8 was a subcutaneous graft.

Following this condition further, and testing the hypothesis that the scrotum has a very definite and decided influence upon the process of spermatogenesis, the writer has carried out an extensive series of testis transplantations in different localities in the body (125). More than one hundred rat testis grafts from intraperitoneal, subcutaneous, intramuscular and scrotal positions were recovered; spermatogenesis continued in many grafts up to the spermatocyte stage and mitoses proved that active division of the germ cells continued. The newly formed cells however did not adhere and produce a complete epithelium but instead they escaped from the epithelium into the lumen to degenerate. Testis grafts, in the scrotum, were many times similar to grafts from other localities but several of the scrotal grafts showed normal seminiferous tubules. These tubules contained a normal, active germinal epithelium, many cells of which were in stages of mitosis and spermatozoa were present in quantities similar to normal testes. In these cases there is no question that differentiation continued in the graft. The testes transplanted were taken from animals two to ten days after birth, at which time the tubules are embryonic in development, and they were transplanted onto the tunica vaginalis of totally castrated males; in sections of the grafts the remains of the old ligature can be seen. These grafts were removed two to six and one-half months after transplantation.

These facts teach us therefore that a mammalian testis graft can develop spermatozoa provided it is transferred to a locality where such is possible. To date the scrotum is the only locality where such differentiation has been obtained, and since it fits in so consistently with the many other elements of proof for the function of the scrotum, we have good reason for suspecting that it will prove to be the only locality in which germ cell differentiation can go to completion.

One of the many debatable points arising in sex gland transplantation has had to do with the possibility of successfully transplanting a gonad into an animal of the opposite sex. Herlitzka (70) and Schultz (165) were to my knowledge the first workers to attempt, with successful results, such transplantation in the mammal. Each, working with the guinea pig, succeeded in transferring the ovary into normal males and recovered later the

ovarian graft. Herlitzka in particular mentions that the ovarian graft is not hindered in its incorporation or persistence by the normal testis of the male host. Basso (9) likewise recovered ovarian grafts from normal male rabbits; they persisted as satisfactorily as when transplanted into females and contained normal Graafian follicles in all stages of development. Marshall and Jolly (106) were able to transplant the ovary into a male rat with limited success. Cevolotto's (35) grafts of the testis into subcutaneous positions in the female rabbit persisted as well as when grafted into males.

Steinach was unable to obtain persistence of a gonad of the rat or guinea pig after its transfer to a normal animal of the opposite sex. Thus he could successfully transfer an ovary into a male animal previously castrated, or a testis into a previously spayed female, but he could not obtain persistence without previous elimination of the normal glands of the host. In 1916 however he was successful in transplanting simultaneously an ovary and a testis into a previously castrated animal.

Around this series of results Steinach constructed his hypothesis of antagonism between secretions from the two opposite sex glands. He believed each gland produced a substance that acted adversely on the opposite sexed organ, and that this influence was sufficiently potent to prevent, for example, the growth of an ovary in a normal male or the growth of a transplanted testis in a normal female. When the environment was neutral, however, as might be conceived to follow elimination of the normal gonad from either animal, then simultaneous transplantation of the two opposite sexed organs into the neutral host would be followed by incorporation and growth of each. Thus he believed that the antagonistic forces were somewhat overcome, that each had an equal chance of incorporation and persistence.

Sand like Steinach was unable to obtain incorporation of the gonad of one sex in a subcutaneous position in a normal animal of the opposite sex; with removal of the normal gonads of the host such could be obtained. However, by transplanting an ovary into the substance of the testis he found that the ovary graft persisted and carried on its differentiation in a normal manner, and the testis likewise remained normal and produced spermatozoa. He thus saw an inconsistency in Steinach's antagonism hypothesis, but there was still to account for, his failures to obtain growth of the graft in other parts of the body without first removing the host gonad. To account for this Sand developed his hypothesis of "atreptical immunity."

In brief, Sand conceives that the body elaborates a specific food substance essential for growth of the sex glands, but a non-sex specific substance that either gland could utilize. This substance, he believes, is rapidly removed from the blood stream and stored within the organ in question. Therefore, he believes that a normal testis would so completely remove the specific substance, storing it within the gonad, as to make it unavailable for the ovary transplanted into a subcutaneous position and this graft perishes because of starvation for it. If however the ovary is placed within the testicular substance this specific food substance would be available to it and persistence follows.

It will be realized, therefore, that both Steinach's "antagonism" hypothesis and Sand's "atreptical immunity" hypothesis were constructed primarily to account for their failure to obtain growth and persistence of gonads transferred to normal animals of the opposite sex. In this

laboratory, however, we have not found the need for such hypotheses. Moore (115) reported successful results from transfer of ovaries into male rats that retained one normal testis, and testes transplanted into females with one ovary present. It was our earlier custom to operate simultaneously on a young male and female of the same age, removing one testis from the male to the female, and one ovary from the female to the male; thus each animal retained one of its normal sex glands and the transplanted gland of the opposite sexed animal. Many months after the operation such ovarian grafts from males with one testis present were found to be carrying on their normal differentiation, from the fact that normal Graafian follicles of all stages of development were present, even to stages showing maturation of the ovocytes; the single intact testis of the male was entirely normal and the animal was utilized as a breeding male. Similarily the testis grafts were recovered months after transplantation from the female whose single ovary remained in place and functional. These testis grafts, for the reason that they were in intramuscular and intraperitoneal positions were not in a normal condition but were entirely similar to grafts from such localities in castrated males. Moore called attention to the superflousness of hypotheses explaining lack of such persistence in as much as no difficulty was exerienced in obtaining persistence of the graft in the opposite sexed animal.

Bohn (21) reviewing Moore's results and contentions saw in the experimental procedure a confirmation of Sand's hypothesis. In as much as he takes for granted the necessity of removal of one sex gland before the gonad of the opposite sexed animal would survive Bohn argues that removal of the one gland would allow sufficient amounts of the specific food substance to be left in the general circulation and the opposite sexed transplant could therefore persist. Moore (120) however pointed out the fallacy of this reasoning by citing preliminary results of later experiments wherein such transplantations were carried out successfully without interfering in any way with the normal intact gonads of the opposite sexed host (see 125). Thus in a large series of testis grafts many of which were transplanted into normal females, such grafts persisted for months and when removed were found to contain seminiferous tubules in active spermatogenesis: the cells produced by mitosis in the germinal epithelium escaped into the lumina of the tubules and degenerated, in all essentials the exact counterpart of tubular activity in grafts located in castrated males. This condition holds, moreover, even during the course of pregnancy. Testis grafts have been removed from at least ten normal females that had completed the period of pregnancy, delivered and suckled normal litters of young and were killed immediately on weaning; the grafts in such cases have persisted for months after transplantation. The same has been found in many other normal females that had not been bred.

Thus it is seen that neither the two ovaries nor even the condition of pregnancy itself has any apparent deleterious influence upon spermatogenesis so far as this function can be consumated in the environment in which the graft finds itself. Grafts made in similar positions in the normal male, normal female, pregnant female, or castrated male appear to have the same capabilities of growth and persistence as well as of differentiation; it is to be remembered, however, that the graft placed in the scrotum can complete differentiation and produce normal tubules containing spermatozoa.

V. THE FUNCTIONAL SIGNIFICANCE OF THE SCROTUM

Preceding sections have directed the attention to some conditions under which a testis fails to continue its normal gametogenetic function. Thus undescended testes of scrotal animals do not produce germ cells and double cryptorchidism is accompanied by sterility; removal of healthy functional testes from the scrotum to the abdomen results in rapid and complete destruction of the germinal tissues, and return of a testis in a degenerate condition from the abdomen back into the scrotum permits of recovery and return to a normal functional condition, whereas a testis intermediate between the two cavities may likewise show an intermediate condition from degenerate to normal; ligation of the vas deferens does not affect the normal function of germ cell differentiation provided the testis suffers no displacement from its normal position by adhesions or other factors unrelated to mere occlusion of the excurrent duct; and finally testis grafts are found capable of producing spermatozoa only when transplanted into the scrotum. In each case, therefore, the common factor is an association of the testicular tissue with the normal scrotal position for the organ. With this information derived from experiments before us, the work in this laboratory was directed to the study of the nature of the causes for germ cell degeneration when the organs are extrascrotal in position. Why do undescended testes fail to develop normally? Why do testes removed from the scrotum to the abdomen undergo rapid and complete germ cell degeneration? Why do testis grafts complete their normal development only when placed in the scrotum?

By experiment all hypothetical factors were eliminated as primary causes of germ cell degeneration except the possibility of higher abdominal temperatures, or conversely lower scrotal temperatures. While experiments were in progress to prove or disprove the differential temperature hypothesis Crew (41) on hypothetical grounds advanced the suggestion that the aspermatic condition of improperly descended testes might be due to higher temperatures in the abdomen than in the scrotum.

Several means suggested themselves to prove or disprove the idea, among which were investigations (1) to ascertain if real differences in temperature did actually exist in the two localities; (2) to determine if prevention of heat loss from the scrotum affected the welfare of the organ; and (3) to study the effect of applying temperatures somewhat higher than normal to the scrotum or testes direct.

a. Scrotal and abdominal temperatures

It is well known from the experiments of Benedict and Slack (11) that a temperature gradient exists in man, from the deeper cavities of rectum and vagina to the external skin, of approximately 5°C. It has also been stated that temperatures within the scrotum were less than in the abdomen without citations of such observations. Moore and Quick (128) however, determined that the interior of the rabbit, rat and guinea pig scrotum was invariably some degrees cooler than the interior of the abdomen. This difference, detectable at ordinary laboratory room temperature, increased as the external environmental temperature diminished. The guinea pig and rabbit scrotal temperature was 1½° to 3°C. lower than abdominal temperatures at a room temperature of 30°C. but this difference was more marked in the rat. Thus in the white rat observed in a room temperature of 16°C. it was found that the interior of the scrotum

was 7°C. lower than the interior of the abdomen.

Thus direct proof is afforded that the actual normal environmental (scrotal) temperature is lower than that of the general body temperature and the hypothesis is greatly strengthened that a differential body temperature is the causal agent in testicular degeneration when the organ is abnormally situated in reference to the scrotum.

b. Scrotal insulation

The fact of a differential temperature in the abdomen and scrotum necessitates the assumption that the scrotum is a local thermoregulator serving to control the temperature around the testes. If such a regulatory mechanism is essential for normal testicular activity it should follow that prevention of the control would be fatal to the active elements of the organ. Moore and Oslund (131) choosing the ram with its large pendent, pear-shaped scrotum as the most suitable animal for experiment, carefully covered the scrotum first with a layer of woolen batting, second with a layer of heavy woolen cloth, and finally with a layer of waterproofed canvas material. Sewing each layer separately so as to closely fit the external scrotal contour it was believed that at least a part of its regulatory function might be done away with. Care was exercised to prevent encaseing materials from sagging upon, or binding, the scrotum; supports over the animal's back allowed the scrotum to hang its full length and at all times a finger could be passed inside the covering. The animal was sacrificed ninety days after the scrotal wrappings were applied, meanwhile running freely in a barnyard. Sections from different regions of the testis showed that the scrotal insulation produced a decided degeneration of these organs. No normal seminiferous tubules were found in the testis nor could spermatozoa be seen from any region of the testis proper. The cells of the germinal epithelium had either been entirely removed or could be seen in stages of degeneration. The lumina of many tubules contained degenerating masses of cells thrown out from the epithelium.

We thus see that prevention of the thermoregulatory function of the scrotum causes an animal to sterilize itself with its own body heat. The germinal tissue destruction is highly similar to that found in experimental cryptorchidism. This result alone would appear sufficient to definitely establish the function of the scrotum as a local thermoregulator, and a scrotal position as indespensible to the normal activities of the testes in those mammals normally developing a scrotum.

c. Heat application

As confirmative proof of the effects of higher temperatures upon the welfare of the testis, artificial sources of heat have been applied externally to the scrotum as well as directly to the testis itself. Moore and Chase (126) reported that external application of higher than normal body temperatures to the scrotum was followed by degeneration of the testes. Discussing the effects more in detail Moore (123) showed that single exposures to water approximately 7°C. above normal body temperature, running over the scrotum, was followed within five to ten days by extreme degeneration of the testes. Graded effects of this influence were obtained by varying either the length of application of heat (using warmed water, electric stove, or electric light) or the degree. Testes suspended in a normal saline solution 6° to 7°C. above normal body temperature for five minutes suffer the loss of practically all the ger-

minal cells within ten days. Similar exposures of testes to saline of a few degrees below normal body temperatures leaves the testis normal. It is the higher temperature, therefore, and not the operative procedure that is the cause of degeneration.

The Japanese worker Fukui (50, 51, 52) determined that testes of the goat, rabbit, guinea pig and rat degenerate rapidly after exposures to artificial heat slightly above the normal body temperature. Fukui employed as sources of heat an electric arc, sun light, hot water, hot air and paraffine. Particular emphasis should be given his findings that a testis elevated to the abdomen requires approximately the same length of time for degeneration as does a testis to which the equivalent of body temperature is applied externally to the scrotum. A second point of particular importance is his findings on artificially cooled cryptorchid testes. In the dog he found that when both testes were replaced in the abdomen and an artificial cooler applied to the ventral abdominal wall in the region of one testis, the testis in the artificially cooled region remained normal while the uncooled one degenerated.

With the accumulated data from so many and varied types of experiment bearing directly on the condition of the testicle we may now regard as proven that temperatures within the abdomen are above the optimum for spermatogenesis. Thus we have an explanation of conditions inherent in undescended testes; we have a partial solution for the behavior of testis grafts; and we see an explanation for perhaps most of the incorrect conclusions on the effects of vasectomy.

d. The significance of the scrotum

Appearing only in mammals, the hitherto unexplained conditions of scrotal formation, and testicular descent, appear in a new light. Thoroughly appreciating the fact that the lowest mammals (monotremata) retain the reptilian and lower vertebrate type of abdominal testes, a survey of mammalian forms forces attention to a gradual evolution of the scrotum and testicular descent. The marsupials, more or less specialized in many ways, are likewise aberrant in scrotal formation in as much as the scrotum is perpenial and of a somewhat different type than that found in other mammals. Many edentates, like the monotremes, retain the testes in the abdomen normally, as do some of the insectivores. Certain members of different lower groups of mammals have a seasonal spermatogenetic cycle at which time the testes are either in contact with a thin area on the abdominal wall or lie in a relatively temporary scrotum. The active production of germ cells is therefore correlated with this simple scrotal beginning (Rasmussen, Tandler and Gross, Marshall, Griffiths, Hansemann, etc.). Recession of the organ from this temporary scrotal position is followed in general by loss of capacity to produce germ cells. Among rodents (rat, rabbit, guinea pig, etc.) the testes usually have passed through an inguinal canal into a scrotum but separation of the latter cavity from the abdomen is not complete; the inguinal canals remain patent throughout life and the testes can be elevated into the abdomen and redescend into the scrotum. Terrestrial carnivora possess the closed scrotum but in some aquatic carnivora the testes remain in the abdomen. The majority of the ungulata show a typical descent into the scrotum but again several members of this group retain the testes in the abdomen (hyrax, elephant, rhicoceros). Abdominal testes also exist in some of the more highly specialized aquatic forms (cetaceans and sirenians) but primates

normally carry the scrotal development to completion and the closed inguinal canals prevent reëntry of the descended testis into the abdomen.

There is thus revealed in general a correlation of scrotal development and mammalian evolution but as in the evolution of so many structures (appendages, teeth, etc.) Variations have occurred. Such an evolution is not necessarily invariably associated with the acquisition of higher body temperatures for the bird, with its slightly higher temperature than mammals, has retained the testes in the abdomen.

Without attempting to explain the cause of such an evolution in terms of one hypothesis or another attention may be directed to the structural characteristics of the scrotum that would fit it for such a thermoregulatory capacity. Primarily an outpouching of the peritoneum against a thin area of the abdominal wall, continued elaboration has produced a sac like structure lined by the tunica vaginalis, a derivative of peritoneum, covered by an extremely thin integument well provided with sweat glands and without subcutaneous fat. Active adjustment to external temperatures can be noted in many animals by the great relaxation of the scrotum in hot weather as against its decided contraction in the cold; within certain limits the amount of relaxation and consequent separation from the body is a function of the external temperature. That it does function to reduce the environmental temperature of the testis has been proven by Moore and Quick, and it is now fully appreciated that such an adjustment is essential for those mammals normally developing such a structure.

Data for a consideration of the possible relationship between degrees of scrotal formation and the body temperature of the animal are not available, but it would appear probable that in many animals lacking the scrotum the body temperature would be of a variable character and subject to wider fluctuations than would occur in those forms where complete descent into a well formed scrotum has taken place. Different degrees of degeneration by abdominal replacement of the testis of rats and guinea pigs correlates with a less restricted range of body temperature. Temperatures against the internal body wall of the rat were found to be a degree or more lower than among the viscera in a deeper position; the variations in this respect are greater than are those found in the guinea pig. Correlated with this fact we find that abdominal testes of guinea pigs are injured considerably more in a given length of time than is the rat testis; guinea pig testes elevated into the abdomen for a period of two weeks are usually more degenerate than the rat testis similarily treated for six months. But yet in the guinea pig, despite the total loss of the generative portion of an abdominal testis in two weeks, a testis graft subcutaneous in position may continue its early stages of spermatogenesis and build an epithelium practically normal in its cellular content for a period of nine months (see fig. 8).

We are justified in concluding, therefore, that in the evolution of mammals a condition has been produced such that the testes cannot carry on their primary function when exposed to the normal abdominal temperature. There has been a gradual recession of the organ posteriorly where first it comes to lie against the anterior abdominal wall in a cooler and primitive scrotal-like area. Carried farther this primitive beginning has gradually become more pronounced and in the higher forms the scrotum with its special structural peculiarities becomes closed off from the abdomen and provides within its interior

a temperature environment lower than that in the abdomen. Along with this newly acquired structure and function the testes of higher mammals depend upon the very conditions that are provided. Without this thermoregulatory function the testes cannot produce spermatozoa. Removed from the cooler environment of the scrotum, or so interfering with the scrotum that it cannot regulate the temperature about the testes, the organ cannot perform its primary function in the general economy of the animal body—that of producing spermatozoa.

VI. THE EFFECTS OF ROENTGEN RAYS ON THE TESTIS

The discovery of the X-rays by Roentgen in 1895 and the accidental effects upon the skin in producing inflammations and dermatitis caused attention to be focused on these, particularly their effects upon the organism or individual parts of it.

The destructive effects of the X-rays on the gonads, more or less accidentally, discovered by Albers-Schonberg (1) has led to considerable experimental work on the reproductive glands. Albers-Schonberg exposed male rabbits and guinea pigs to X-rays and failed to obtain offspring in subsequent matings with untreated females; sterility was due to a lack of spermatozoa in the testes. A few months later Frieben (49) reported that the seminiferous tubules were reduced in size, and that the lumina of the tubules often contained loose cells in a state of degeneration; there was an entire lack of mitoses in the cells of the tubules.

Brown and Osgood (30) reported that the mere presence of men in an X-ray room, either as operators or helpers, led to an azoospermic condition. Phillip (138) similarly reports that X-rays cause sterility in men. One, a tuberculous individual, voluntarily submitted to daily exposures of from ten to fifteen minutes for thirty days. Whereas motile spermatozoa were obtained for a considerable time after the beginning of the exposures it was found at the end that the testes were entirely devoid of spermatozoa (hypodermic syringe sample); under the limits of the observation the individual reported no diminution in sexual capacity, despite his sterility. In 1904 and 1905 Bergoine and Tribondeau noted that X-rayed testes of the white rat were rendered superficially translucent, and that fluids issued from any cut surface of the organ. Later this edematous condition disappeared and the seminiferous tubules were found to lack a germinal epithelium, or at most contained only a few spermatogonia. The degree of destruction in the testis was roughly proportional to the X-ray dosage.

These writers noted a high degree of selectivity of the X-rays for the germinal portion of the gonad and that Sertoli cells appeared unaffected; the latter were not only present after all germ cells had been eliminated but were increased in number through amitotic divisions. They state that Sertoli cells may thus multiply and increase in number but are incapable of producing cells of the germinal line. Three months after the last exposure to X-rays they were unable to find any germinal cells in the testis; the interstitial cells were overabundant. They believe the X-rays act upon the individual cells directly; that rapidly dividing cells are more easily affected; and that spermatozoa are unchanged by such exposures. Indeed they determined that human spermatozoa exposed directly to the rays for thirty minutes suffered neither change in form nor loss of motility. A single exposure of the rat testis was sufficient to render the animal sterile.

Villemin (1925) confirmed the main

contributions of Bergoine and Tribondeau. Guinea pig testes exposed to X-rays suffered complete destruction of the seminal tissue but interstitial cells were unaffected; such animals retained their sexual ardor. Villemin determined that a smaller dosage was followed within three and one-half months by regeneration of the testis and production of spermatozoa. He maintained that as long as spermatogonial cells remained in the degenerate testes recovery of normal function was possible, but without spermatogonia no recovery could take place; Sertoli cells were incapable of producing the cells of the seminal line.

In a series of papers Regaud and Blanc (145), Regaud and Dubreuil, (146, 147) Regaud and Nogier (148), the earlier studies on X-ray effects on the testis were confirmed and extended. Exposures of young rabbit testes before differentiation did not subsequently alter testicular development, whereas in the differentiated testis complete sterility was produced; but despite sterility there was no diminution in sexual ardor. Those receiving X-ray exposures copulate and at first discharge motile spermatozoa, but these appear incapable of fertilizing ova; later, spermatozoa were absent from the semen.

Subsequent X-ray work on the testicle by Harvey (67) Herxheimer and Hofmann (71) Wakelin Barratt and Arnold (199), Hewer (72), and Ancel and Bouin (4) give added confirmation of the selective, destructive action on the germinal portion of the testes. Simmonds (171) determined that mesothorium acts similar to X-rays. Wakelin Barratt and Arnold describe intimate changes in the individual cells following exposure. All workers are in agreement that selectivity is the outstanding event. Cells of the seminal line show no changes for the first twenty-four hours after exposure; subsequently the protoplasm becomes somewhat cloudy and approaches a general aspect of coagulation; nuclei, especially those in mitosis, show many abnormalities. In general two different types of disposition of the germinal tissue is observed. First there is a local cytolysis of cells *in situ* leading to a liquefaction of material and the consequent vacuolated appearance of the epithelium. The products of cell cytolysis are absorbed into the blood stream. Accompanying this condition there is a second one in which many or most of the remaining cells are sloughed into the lumen of the tubule. Such masses of material contain cells little modified, cells showing degeneration changes, and a considerable mass of debris from cells that have undergone partial solution or fragmentation. Some of the cells thrown out into the lumen may be carried into the epididymis where their further degeneration and absorption can be followed.

The type of behavior in the X-rayed, degenerate testes is highly similar to the type of degeneration encountered in testes confined in the abdomen. Cell changes, the local cytolysis, sloughing into tubules, transportation into the epididymis, the retention of spermatozoa in the vas deferens are all common conditions in degeneration from the two different causal agents. The selective action on the seminal line and the retention of Sertoli cells and interstitial elements are common to the two types. Shrinkage of the seminiferous tubules and the apparent interstitial hypertrophy are entirely similar. Finally the powers of recuperation of the testes from spermatogonial elements gives a series of conditions that would make it almost impossible in a study of degeneration to decide between the two causal agents. These similarities force themselves on our attention as well

as the fact that the real causal effect in each case is not apparent. We know neither the ultimate cause of degeneration as produced through testis elevation to the abdomen or the external application of higher than normal temperatures, nor the actual ways in which X-rays produce their effects. It may ultimately be proven that the similarities are more than superficial and that the two causal agents may have a fundamentally similar action upon the protoplasm of this labile tissue.

VII. HYPERSENSITIVE REACTIONS IN THE TESTICLE

An interesting testicular reaction but recently discovered, and bearing so great a similarity to reactions heretofore considered, is that used by E. R. Long and his student. Long (97) determined that guinea pigs experimentally infected with tuberculosis of mild virulence intraperitoneally or in the axilla, twenty days or longer, show a most striking reaction when one testis is reinoculated with tubercle bacilli. Within twenty-four hours the inoculated testicle becomes swollen and extremely hyperemic and edematous. Sectioned, the testicle shows marks of degeneration of the germinal tissue and within one month practically all germinal cells are destroyed. The opposite uninjected testicle, or an injected testicle in a nontuberculous animal, does not exhibit the inflammatory reaction or degeneration. Carrying the observations farther Long showed that intra-testicular injections of tuberculin (0.01 cc. O.T.) into previously mildly infected animals was similarly followed within twenty-four hours by great swelling, edema, hyperemia, and early sloughing of the germinal portion of the seminiferous tubules; within four weeks after such injections all germinal cells with the possible exception of spermatogonia have been removed from the organ. Along with the tubular degeneration ".... the interstitial cells (of Leydig) have proliferated in a striking manner." "At this period (24 hrs.), when the testicle of the previously infected guinea pig is so highly inflammed, the testicle of the noninfected one shows little or no change either grossly or microscopically. Tuberculin, so destructive to the testicle of the infected guinea pig, is absolutely nontoxic for the testicle of the normal animal." Spermatocytes and spermatids appeared the more susceptible, spermatogonia and spermatozoa more resistant. Similar reactions to other hypersensitizing agents are reported by Long and Seyfarth (98) and Seyfarth (168).

In these conditions it is clear that we are not dealing with a primary infection in the organ but with a reaction between substances produced in an organism from an infection and some substance of an antibody nature. The reaction produced between the two substances is therefore to be considered the causal factor in destruction of the germinal elements. Since the marked inflammatory reaction occurs within such a short time it appears that we may have to deal again with a temperature factor.

VIII. TESTICULAR REACTIONS UNDER OTHER CONDITIONS

Sickness. It has been known at least since 1882 that often the testes become aspermatic in certain types of illness whereas in other types, even chronic conditions, the spermatogenetic function may continue unimpared. Busch (32) reported on the spermatozoon condition of the testes of one hundred autopsy cases. In all cases of sudden death, whether through accident or suicide, the testes were found to contain spermatozoa. In acute cases (not over four weeks duration of sickness) 15 per cent showed a lack of

spermatozoa, whereas in longer sicknesses (complicated pulmonary infections, pneumonia, etc.), 31 per cent of cases lacked spermatozoa in the testes. Hansemann (68) studied the testis of human autopsy cases and determined that often the testes have been deprived entirely or partially of the germinal portion following pneumonia and typhus fever; in tuberculosis certain lesions may produce localized destruction but in the unimpaired portions spermatogenesis has continued. Cordes (38) examined the testes from human atutopsies after common diseases such as pneumonia, scarlatina, peritonitis, meningitis, etc. Many of those dead of relatively rapid pneumonia or other febrile conditions lacked spermatozoa but retained a fairly well defined germinal epithelium. In general, the longer duration of the common diseases was accompanied by the greater testicular degeneration; in many cases there was complete loss of the germinal cell line with the possible exception of a few scattered spermatogonia. Mills (110) and Wolbach (211) studied testes obtained from autopsy of army recruits dead in camp from influenza and pneumonia. Mills in particular follows out as closely as he was able the correspondence of the testicular condition and the type of affliction; many of the cases were complicated with measles, epidemic influenza, etc. but he states that "roughly the severity of the injury corresponds directly with the duration of the pneumonia as closely as with any other single variable factor." The injury noted consisted of the disappearance of various cells in the epithelium of the seminiferous tubules, sloughing of the epithelium into the lumen with cellular destruction, and in more severe cases all germinal cells except spermatogonia had been removed from the tubules. Mills makes a futile attempt to correlate the different types of degeneration with a common cause such as pure infections, complications, durarion of infection, or with the infectious organism involved, and comes to the conclusion that "In the absence of definitive evidence to the contrary, the cause is assumed to be circulating toxins . . . ."

At the time of this writing the effects of higher temperatures in causing rapid degeneration of a similar nature in perfectly healthy testes was not known. It appears highly probable, from our experiments with higher temperatures, that the mere febrile condition alone is sufficient to produce all the types and grades of degeneration described. This appears all the more probable in view of the close correlation between the duration and the state of degeneration; rapidly fatal infections are in general correlated with testicular injury of a lesser grade than in more prolonged sicknesses. It is believed that this common explanation (high temperatures) for so many different cases of germinal epithelium destruction can be invoked here as the real cause of testicular degeneration following febrile sickness. It is well known that patients recovering from severe and prolonged fevers of different kinds are not rendered permanently sterile. This is in keeping with the general findings that where spermatogonial cells survive the destructive effects, complete regeneration of the testes follow.

Alcohol. It has been shown in many cases that the germinal tissue of the testicle of man and experimental animals is very susceptible to injury by alcohol when no perceptible injury is detectable in other organs of the body. Busch (32) noted that confirmed drunkards often showed a diminished amount of spermatozoa or an entire absence of them, particularily so when alcoholism was accompanied by cirrhosis of the liver. Simmonds (170) found an azoospermic condition one

hundred and twenty-five times among one thousand human autopsies. Of this number 7 per cent were cancerous, 14 per cent afflicted with chronic nervous disturbances, 18 per cent with phthisis, and 60 per cent were chronic alcoholics. He determined that among all autopsied human males there was a greater number sterile from the use of alcohol than from venereal diseases. Bertholet (16), and Weichselbaum and Kyrle (200) likewise show that testes of chronic alcoholics are degenerate; histologically different grades of injury have been illustrated by the latter writers. Degeneration from the use of alcohol is quite similar to the type found after elevation of temperature; spermatocytes and spermatids are often found loose in the lumen of the seminiferous tubule undergoing degeneration. Other tubules have been described as consisting of a vacuolated epithelium apparently caused by degeneration and liquefaction of cells locally in the meshes of the Sertoli reticulum. More severe injury involves all germinal cells with the exception of a few spermatogonia. Bertholet found that only two men out of thirty-nine habitual drunkards autopsied failed to show some degree of testicular degeneration.

The interstitial cells in alcoholic degeneration have been described as hypertrophied and in some cases as little changed. In general the testes are somewhat more firm and usually slightly smaller than normal. The seminiferous tubules are often of smaller diameter than normal ones.

Bouin and Garner (26), Arlitt and Wells (5), Allen (3) and Kostitch (79) have determined that feeding alcohol to normal rats causes the testes to show degeneration changes. There is a general agreement among these writers that degeneration follows in the reverse order of production of the different elements of the germinal line; thus spermatozoa are often absent from testes that contain a fairly normal germinal epithelium. More severe injury is to be associated with both a sloughing of the spermatocytes and spermatids into the lumen, or a local cytolysis in situ and the consequent vacuolated condition of the Sertoli syncytium. The usual condition, perhaps, is to find some spermatogonia that apparently have not been affected.

With experimental alcoholism, as in chronic alcoholism in the human, the interstitial tissue is reported both as greatly hypertrophied and as practically normal.

Under the influence of alcohol, therefore, we find testicular degeneration of similar grades to those effected by high temperatures. It is a question whether there is any relation between the action of the causal agents in the two conditions but each serves to emphasize the labile nature of the cells of the reproductive line. The possibility of regeneration from uninjured spermatogonia is likewise common to the two conditions.

Dietary deficiencies. Allen (3) studied the testes of albino rats reared on a diet deficient in the water soluble vitamine (Osborne and Mendel). Rats that were otherwise normal had experienced degeneration of their germinal tissues. A usual condition was to find an entire lack of spermatozoa but in some of the animals the effects had been so severe that all cells of the germinal line have been reported absent, the seminiferous tubules containing only the Sertoli reticulum. At times the interstitial tissue was increased and in other testes approximately normal.

Mason (107) studied the testes of white rats after feeding with a basic diet sufficient to maintain a normal growth curve and good health of the colony. After the

attainment of sexual maturity male rats fed on the diet experienced a progressive degeneration of the germinal tissues. Spermatozoa were the first cells affected and these were followed in turn by spermatids and spermatocytes. Usually the spermatogonia were retained along with the Sertoli tissue. The interstitial cells appeared to remain normal. When to the stock diet, a small amount of lettuce was added the testes were unaffected. Mason consideres the cause of degeneration to be a lack of Vitamine X (Evans and Bishop). He states that recovery from degeneration does not follow within seventy-five days after placing the animals on a normal diet. If spermatogonia are present, however, and uninjured, it would appear from other considerations that recovery should follow on return to normal diets.

We see therefore that the testis, in reference to its germ cell differentiating capacity, is an extremely sensitive organ. Many other conditions, not considered here, would only serve to emphasize its labile nature. There is yet much to be learned in its biology but it is evident that considerable progress has been made. Cross currents in the advance of our knowledge concerning the gametogenetic function are confusing but having considered a few of the major trends in this advancement we now come to consider the testis as an organ of internal secretion.

IX. INTERNAL SECRETION OF THE TESTIS

General considerations. It has been thoroughly appreciated for centuries that removal of the testicle from man or animals has an effect upon the entire organism. The present day custom of castrating domestic animals is an old one, and the Eunuch is referred to in biblical literature. It is clear that in general the testicle exerts some influence upon the character of the animal that leads to the production of a more or less well defined masculine type. In domestic animals this is at times indicated by characteristic body form; in some ruminants horn growth is a specific male character. In man body form is not always clearly indicative of masculinity, but certain skeletal structures such as the pelvis is usually quite specific in form; growth of the larynx and the attendant coarseness of voice is relatively diagnostic; hair tracts on the body of man differ to some extent from those of woman, and within somewhat narrower limits the growth of beard and mustache on the face. The entire accessory organs of reproduction in the male are dependent upon the testis for their complete development; penis, seminal vesicles (where present) vas deferens, and prostate remain characteristically infantile when the testes have been removed at an early period in life. In both man and animals testis removal has been held to favor growth in length of the long bones (69, 141, 167, 186) due to delay in ossification of the epiphyses; many cases of overgrowth of bones following castration have been reported but in the guinea pig such differences if present at all are minimal (117). Finally the psychic nature or the attitude of the male to the female in both man and animals appears to be quite largely conditioned by the sex glands, though it appears to be a question whether they are wholly dependent upon testicular influences. It may be suggested that in attempting to define the testicular influence upon both somatic and psychical characteristics many writers have allowed their ardor to carry them into dangerous territory and have added confusion to the problems rather than restoring order.

Steinach (177 to 183) in his celebrated work of hormone analysis in rats and guinea pigs has used as indicators of

masculinity certain types of hair coat, fat deposition, size, weight, and the psychical nature. Moore (112, 115, 116, 117) has criticised these criteria on the grounds that they are not sufficiently diagnostic to enable one to choose between normal males and females in a mixed colony, and are therefore questionable indicators of sex. Such characters as are not sufficiently well defined or characteristic are dangerous implements with which to attempt to advance our knowledge of the internal secretion of the sex glands.

Such misleading applications of questionable indices is nowhere more apparent than in the clinical literature concerning sex and the effects of supposedly remedial operations such as vasectomy and testis transplantation. For the purpose, one may assume that a man of fifty-five to sixty years of age is prematurely senile due to slowing down of the activity of his sexual apparatus; cases are on record where lack of spermatozoa in the semen is considered adequate proof of senility. Under these conditions hospitalization embodying rest and excellent care, combined with a vas deferens ligation, or a testis transplantation, and plenty of suggestion and anticipation, have produced most remarkable results particularily in reference to the reassumption of sex inclinations; spermatozoa may again be found in the semen. Busch (32) in 1882 points out that men of eighty years may possess functional testes producing spermatozoa and likewise men above ninety years. Many conditions may lead to a temporary azoospermy but as long as spermatogonia remain capable of activity the entire seminal line can undoubtedly be restored if the unfavorable causes, whether mental or physical, be removed. Rest, good care and psychology work wonders along with, perhaps, a harmless operation, but the operation is all too often credited with the entire improvement.

Castration in man undoubtedly has a great psychical effect upon the individual but certain observations if rightly considered should temper our conclusions that the entire psychical reaction is conditioned by substances poured into the body from a testis or a graft. In 1801 Sir Astley Cooper (39) removed the second testis from a man semicastrated two years earlier. Cooper following this case for twenty-nine years, reports the patient experiencing an emission on the third night after operation. Similar observations have been reported following testis transplantation with the implications that the effects of the testis transplant have this early become detectable. During the first year after total castration Cooper's patient experienced emissions in coitus. Two years after operation erections were strong but relatively transient. A general decline was noted, but twenty-eight years after the removal of both testes erections were experienced as well as erotic dreams and sensations of emissions. It is likewise instructive to follow the reports on the psychical nature of the Skopecs studied by Tandler and Grosz (186, 187, 188). This religious sect, practicing castration of young males, are in general of two types: one grows to excessive height and are lean individuals whereas the second is characterized by excessive fat deposition. The voice is characteristically boyish and it is well known that many famous male choirs and soloists are individuals castrated for the purpose of producing a voice of a particular caliber. These Skopecs, according to Tandler and Grosz, appear to be a long lived group of people, which would appear to minimize the contentions of certain rejuvenation advocates that the life of an animal can be prolonged by vasectomy or testis transplantation. Like Cooper, Tandler and Grosz maintain that sexual desire is not entirely abolished by total castration.

They report one individual totally castrated for twenty-one years, as exercising coitus almost daily and with slight emissions. Erections were strong but not greatly prolonged. It is likewise reported that those upon whom the "Great operation" had been done (removal of both testes and penis) often find pleasureable associations with females and actually experience a type of sexual orgasm.

From such considerations, therefore, it would appear that conclusions from operation on men have not always been stated with caution and much of the literature dealing with the effects of sex gland manipulations cannot be highly regarded.

Work on the effectiveness of sex glands in the experimental animal is likewise often misleading because of a lack of appreciation of the limitations of indices employed to grade the effectiveness. It is usually considered that a male animal shows no inclination towards females after the removal of its testes, but Moore (116) reported castrated male guinea pigs carrying ovarian grafts sufficiently effective to modify the mammae of the male, still acting strongly the part of the male. Indeed he has observed male guinea pigs castrated at thirty days of age that continued to show strong male reactions in the presence of females for sixteen months; the male sex call, pursuit and attempts at mounting the female were so positive that the animal was employed as a test animal to detect females in heat.

Source of the internal secretion. Admitted that the testis does exercise an influence upon the organism that results in the production of the specific male characteristics, the attention of many workers has been directed toward the localization of the source of such an internal secretion.

The work of Berthold (17) gives a starting point in such analyses. He determined in the bird that the testis could prevent the animal from becoming a capon after its removal from the normal to a foreign position if it became vascularized and persisted; the testis placed in the abdomen and supplied by blood vessels from the intestine kept the cock normal for some months whereas complete removal produced a capon. He believed the active principle involved was a substance liberated into the blood stream that exerted a gradual influence over the entire body and that nervous transmission from the testes was not involved.

It was perhaps logical to consider that the generative portion of the testis was responsible for the production and maintenance of the secondary sex characters as well as for the production of mature germ cells. Brown-Sequard (31) on the assumption that an active functional testis was responsible for the health and vigor of the young, injected subcutaneously into himself an extract made from the young testis of dogs and rabbits. At the beginning, a very feeble old man, he believed that he derived great benefits from such injections. He maintained that his muscular strength, vitality and youthful ire were stimulated and that he was able to continue his scientific work for many years longer than he otherwise would have been capable.

It was appreciated long before this time, however, that man and animals with undescended testes retained their virility despite the consequent sterility (Goubaux and Follin (57), Godard (54), Monod and Arthaud (111)). Griffiths (59) is remarkably clear in calling attention to the fact that the general influence of the testis on the animal is entirely independent of its spermatozoon producing capacity.

Leydig (86), in his comparative anatomy studies on the different mammalian testes, described for the first time certain

large cells containing fatty spherules and pigment, as a constant occurrence in the connective tissue stroma between the seminiferous tubules. The significance of these cells, commonly known as the interstitial cells of Leydig has been a point of contention for many decades (Jacobson, Lubarsch, Reinke, Stieda, Plato, von Lenhossek, Bouin and Ancel, Felizet and Braca, Regaud and Policard, etc.). These cells have been studied in a large number of mammals and though a constant feature of the testis they have been found to vary greatly in amount, distribution, and types of cellular inclusions. They appear in relatively large quantities in the embryonic testis, in apparently diminished quantities as the testis develops, but present throughout life, and again somewhat more numerous in an old animal, or man (Plato, Bouin and Ancel, Kasai, Allen, Whitehead).

Among the older writers some were of the opinion that the interstitial cells represented merely an embryonic rudiment left in the testis during its formation and had no other significance, but later worker came to view the elements as modified connective tissue cells having some particular functional significance. Von Ebner observed that the enclosed pigment was more pronounced in older than in younger animals. Jacobson (76) noted that in pathological conditions the interstitial cells were apparently increased in amount while Hansemann (68), in the hibernating marmot, noted that Leydig cells were not in evidence but that two months after hibernation the testis presented the picture of a sarcoma due to the tremendous increase in the amount of Leydig cells; under pathological conditions, likewise, he noted great variation in the amount of these cells.

In attempting to determine the significance of the interstitial cells the current ideas of the time centered around the conception of the Leydig cells as a trophic organ responsible for the nutritional requirements of the generative tissue of the testicle. Plato (139, 140) representing this type of opinion, believed that these cells, located between the blood vessels and the seminiferous tubules, extracted materials from the circulation, elaborated the fat and pigment (which in general he considers only different phases of the same process) and passed these substances through the basement membrane into the seminiferous tubules were he attempted to trace their distribution from the Sertoli cells to the different cells of the germinal line. He figures small canaliculi through the basement membrane containing a stream of the elaborated material from Leydig cells into the tubules.

A year earlier Reinke (150) described elongated, blunt, crystalloid rods in the interstitial cells of a tuberculous human testicle. These he found in normal human testes from the fifteenth to the sixty-fifth year and believed they represented the elaborated products of the cells. He believed the Leydig cells secreted these bodies and from materials detected in the lymphatics concluded that the materials were discharged into these channels, mildly suggesting that they might represent a product having to do with the sexual ardor of the male.

Many different inclusions such as acidophile and basophile granules, crystalloids, fatty spherules, different pigments and other types of stained materials have been described from different mammalian testes and at least until 1903 the general opinion was held that these substances represented the secreted products destined for nutrition of the seminal portion of the testis.

In 1903 Bouin and Ancel after an elaborate histological, cytological and experimental group of researches brought

forward a new point of view regarding the significance of the interstitial cells that has stimulated renewed interest in the entire field of sexual biology. Their own detailed cytological studies (22) and those of their predecessors appeared to establish beyond all doubt that the interstitial cells were glandular in nature and function. Admitting, with many of their colleagues and predecessors, the probability that the secretion products of these cells were nutritive substances for the germinal cells, they believed that all of it was not so utilized and that fundamentally the interstitial portion and the generative portion of the testis are independent tissues. They point out: (1) that the interstitial cells are to be found in intensive secretory activity long before the activity of the germinal portion is initiated. (2) This interstitial tissue is present throughout life in all mammals, and is of a common secretory type though differing in minor details as to inclusions. (3) After the germinal portion has ceased to be active in old animals the interstitial cells continues to secrete. (4) They believe that the primary distribution of the Leydig cells is around blood vessels rather than in close opposition to the basement membrane of the tubules. They trace secretory products discharged into the lymphatics rather than into the seminiferous tubules. (5) In cryptorchid testes there is no germinal tissue whereas Leydig cells are present in an overabundance and actively secreting. (6) After certain diseases the generative portion has suffered degeneration but intersitial cells are retained and are actively secreting. (7) They maintain that occluding the vas deferens causes a loss of all the generative portion of the testis but a retention, perhaps an increase, of interstitial tissue.

From these and other considerations they argue that since there is no interdependence between the interstitial tissue and the germinal tissue, and that since an animal may loose all its generative portion without showing any signs of castration, there must be other tissues in the testis than the germinal line that are responsible for the maintenance of the secondary sex characters. Their histological and cytological studies failed to show any secretory activity in portions of the testis other than the interstitial cells and they therefore conclude that the interstitial cells manufacture a substance that is poured into the general circulation—and internal secretion—which is responsible not only for the production of the secondary sex characteristics but at the same time for their maintenance; the aggregate features of maleness are therefore conditioned by the secretions from interstitial cells alone and this function is separate from, and independent of, the production of germ cells. We now know that their conception of the effects of vas deferens occlusion were wrong (see section III) but the fact remains that by whatever means the condition is brought about, an animal will retain its morphological secondary sex characteristics and its sexual ardor in the absence of at least all cells of the germinal line with the possible exception of spermatogonia.

The internal secretory activity of the interstitial cells had been mildly suggested by earlier workers (Reinke (150), Regau and Policard (149)) but it remained for Bouin and Ancel to develop the idea into a well formed hypothesis. Extending the conception by experimental and morphological studies (24, 25) they have attempted to correlate the amount of interstitial tissue with graded sex differentiation. Thus by removal of a normal testis from the pig, with one testis retained in the abdomen, they believed it demonstrated that the interstitial tissue

of the remaining testis undergoes a compensatory hypertrophy—an approximate doubling in amount of interstitial tissue—to restore the loss conditioned by removal of the descended testis. In cryptorchid pigs they make an attempt to correlate degrees of differentiation of the reproductive system and the interstitial cell content of the testes. They, furthermore, carry the conception to the extent of making the interstitial cells responsible for the determination of the sex of the primordial germ cells.

A tremendous stimulus to the conception of the interstitial tissue as the internal secretory portion of the testis was given six years later by Steinach (177 to 183). The work also of Sand (157 to 164) and Lipschutz and his students (91 to 95) has added a great deal of confirmatory evidence and new observations.

Steinach studied principally the effects of castration and subsequent gonad transplantation in both sexes of the rat and guinea pig, and the effects of ligation of the vas deferens in the male. He showed that castrated males in which testis grafts had been growing retained all their secondary sex characters instead of reverting to merely castrated animals. Since the graft tissue consisted primarily of Leydig cells he considers these to be the source of the sex hormones; these cells were not only retained but were believed to have undergone a compensatory hypertrophy. The seminiferous tubules were always degenerate and contained only the Sertoli reticulum; cells of the generative line were always lacking. Similarily, in ligating the vas deferens, Steinach reported that the generative portion suffered degeneration and in some manner the operation stimulated increased development of Leydig cells. These cells, believed thus to produce an excess of hormone, were then indirectly responsible for stimulating the entire organism and, according to his account, the testes of senile animals previously devoid of germ cells would again become active and a renewed germinal cell activity would follow. The animal was believed to have been rejuvenated, thus becoming a younger animal in all its characteristics. Such operations, largely due to Steinach's reports, are now being employed as remedial measures both on experimental animals and on man (Steinach (183) Sand (163), Benjamin (12)). The experimental work of Sand is largely confirmative of Bouin and Ancel and Steinach. He maintains, with Bouin and Ancel, that unilateral castration causes compensatory hypertrophy of Leydig cells in (experimental) cryptorchid testes.

The experimental work of these biologists stimulated anew the study of the histological structure of Leydig cells with attempts to find the specific secretory products. Whitehead (202 to 208) has described many types of apparent secretory products in Leydig cells of different mammals. Fatty granules or vacuoles appear in most testes but in a few the fatty material is almost wholly absent. Pigment materials of many varieties have been noted and granules of a protein nature as well as crystalloids have again been brought to our attention. Wagner (197) has recently reviewed the literature and added observations to this field of work. He is convinced that the cells are secretory and that the products can be traced into the lymphatics; he believes the individual cell is not destroyed in liberating the secretory products (see also Cejka (34)).

Added correlation between hormones and interstitial cells is found in the study of the free-martin by Lillie and his students (88, 89, 90, 7, 36, 210). The free-martin (a female calf cotwin with a

male) is modified sexually when by a fusion of the embryonic membranes an intermixture of blood, from the male and the female in utero, is permitted. The effective modification is detectable at an early stage and has been found to be effective just after the appearance of Leydig cells in the embryonic testis (Lillie and Bascom (90) Bascom (7)). The effectiveness of the modification is so great that a determined female may be caused to produce testes out of undifferentiated ovaries (Lillie, Chapin, Willier, Bissonnette).

Thus there has developed a relatively strong case in favor of the interstitial cells as the source of the internal secretion, or hormone, of the mammalian testis (the case is somewhat similar in other vertebrates). But if we examine critically the main points of contention it will become apparent that the question is by no means definitely settled.

1. The experiments on vas deferens ligation (see section III) from the morphological side may now be ruled out. It has been bountifully shown that occlusion of the vas deferens in the dog, guinea pig, rat, rabbit, sheep, cat, pig and man causes neither degeneration of the generative portion of the testis nor hypertrophy of the interstitial cells. What, then, of rejuvenation by this means (Steinach, Sand, Haire, Schmidt, Benjamin)?

The criteria employed in experimental animals are of such a nature and so variable among individuals as a whole that it is capable (by selection) of being applied either for or against the hypothesis; and certainly it is selection to choose two positive cases of degeneration as against thirteen negative cases and yet hold that the operation gives positive effects (159). Among animals there may appear certain lassitude, loss of hair, sex disinclination, loss of weight and combative ability in old age but it is not so clear that such conditions are due to lack of sex hormones. This is all the more confusing for the hypothesis when so many have stated that interstitial cells are much more abundant in testes of old animals and man. The same decrepit indicators occur many times in males, of animal colonies with both testes present, that are much younger than the average sex life of the animal. Good care and particular attention often restores such animals to sleek coated vigorous animals. In short, nutritional disturbances, parasitism, and general infection may produce these symptoms. Steinach himself has discussed the inherent difficulties of properly judging an animal a fit candidate for rejuvenation. In man, likewise, some of the most remarkable effects of rejuvenation through vas deferens ligation have been reported on individuals under fifty years of age (see Haire (63), Schmidt (166)) whereas many men are sexually active for much longer periods (see Busch '82). It is merely granting an assumption to accept the statement that such individuals are prematurely senile due to a dimunition of their testicular secretions. No one has yet proven that an individual castrated early in life is shorter-lived or more decrepit than a man in possession of his testes. Indeed Tandler and Grosz (187, 188) maintain that the Skopecs are a long-lived group of people.

It is unnecessary to enter into a discussion of the effects of suggestion and psychology in general, on matters relating to sex in the human individual. It is a condition of mental attitude which in our modern civilization is useless as a qualitative or quantitative indicator of effectiveness of operative procedures (see section IV). Vasectomy, as a remedial, rejuvenating procedure has failed to justify its utilization. Moreover the very basis of the conception has been proven false.

2. Compensatory hypertrophy of the

interstitial cells has been one of the chief objective conditions that has led to the contention that in this tissue resides the source of the internal secretions of the testis. Bouin and Ancel note an apparent doubling of this tissue in a unilateral cryptorchid testis if the opposite one had been removed earlier. They believe this increase compensated for that lost in removal. Steinach claims that hypertrophy occurs in all testis grafts. Practically all writers on cryptorchid tests maintain that this tissue has been increased. We may therefore inquire, first, whether compensation is necessary and the tissue reactions of any significance from the internal secretory viewpoint and, second, whether there is an actual hypertrophy of the Leydig cells.

It is well known that the loss of one testis leads to no diminution of the secondary sexual characteristics in animals or man. Dspite the contentions of earlier writers that a single remaining testis does become larger than normal Lipschutz (92) holds that attainment of ultimate size is only more rapid and that in the end neither generative nor interstitial parts are in any way increased above the normal. Furthermore Lipschutz and his coworkers (95) have proven that 1 per cent of the normal testicular mass is sufficient to maintain all the secondary sexual characteristics. These facts lead one to question the significance usually attributed to the compensatory hypertrophy where such a condition actually follows in single or double cryptorchid testes. If 1 per cent of the normal testicular mass is all sufficient for the animals' need, of what significance is interstitial cell hypertrophy *in each of two complete testes that have remained in the abdomen?*

Hypertrophy of Leydig cells has been held to exist in cryptorchid testes, cases of obliterated vas deferens, testis grafts, after X-ray treatment, excessive temperatures, use of alcohol (chronic or experimental), diseases of many kinds, dietary deficiencies and other conditions. Can such conditions have any significance for the internal secretory needs of the animal? Is it really true that hypertrophy occurs? In brief, practically any condition leading to loss of the generative cells is accompanied by apparent Leydig cell hypertrophy. This may be true for the testis as a whole or minor local portions of it (Kyrle (82)), yet these cells are rarely noted in mitosis. When the germinal epithelium is lost from the seminiferous tubules these latter characteristically decrease in size (122). With a decrease in diameter of the tubules the testis as a whole becomes appreciably smaller and if there were actually no increase in the number of Leydig cells they would naturally be restricted to a smaller area and thus exist, *relative to number of tubules*, in an overabundance. In a given microscopic field therefore, there would be a great increase in Leydig cells. Determination of hypertrophy has been made in this manner and it must be emphasized that a quantitative increase in a given microscopic field is no criterion of the actual content in the entire organ. This question has been discussed in another place (Moore (122)) and it is considered that compensatory hypertrophy not only does not exist but also that any slight increase in number or size of Leydig cells loses its significance as being a quantitative indicator of the internal secretion of the organ. Quantitative methods carefully employed should be a great factor in settling the present controversy of compensatory hypertrophy of the cells of Leydig. The commendable attempt of Bascom (8) will undoubtedly give us much more exact information than has hitherto been available. In contrast to Bouin and

Ancel, Hanes, and Sand, Bascom, using his quantitative method, maintains that there is not an hypertrophy of Leydig cells in unilateral cryptorchid testes after removal of the opposite one.

In testis grafts, contrary to Steinach and Sand, it has been found that, more often than not, there is a lack of apparent hypertrophy of the Leydig cells (Moore (125)). Apparent increases may occur in these grafts but it occurs just as readily in grafts residing in normal females, where with both ovaries present there cannot be a question of the need of increased secretion, as in castrated males. Such increases were found without any definite relation to any secretory need or sexual condition of the host animal. In castrated males, for example, grafts may show large interstitial areas, one graft revealing a large group of typical Leydig cells and another relatively devoid of them, having instead ordinary connective tissue cells between the tubules. It cannot, therefore, be accepted in principle that Leydig cells undergo hypertrophy in testis grafts.

3. The seasonal cycle of the testes shown by some hibernating animals breeding but one a year reveals striking changes in the different tissue of these organs. Hansemann (68) observed that the hibernating marmot had only slight amounts of connective tissue between the seminiferous tubules and no typical Leydig cells; spermatogenesis was in abeyance. Two months after the awakening period, in the height of its breeding season, Leydig cells were so large and numerous as to give to the testis the appearance of a large-celled sarcoma. Could it be that their activity here was definitely responsible for the occurrence of the seasonal cycle? Ganfini (52a) stated that Leydig cells were not absent from the testes of this animal but were only much smaller during hibernation. Regaud (144) found in the mole a different relationship of the testis elements; active spermatogenesis was not accompanied by interstitial cell hypertrophy. After the breeding season the interstitial cells become maximal and so remain after the disappearance of the generative portion; this is subsequent to the breeding season. Tandler and Grosz (189) likewise note that the testes of the breeding mole have few interstitial cells. As the generative portion receds to its minimum development the interstitial cells hypertrophy to their maximum development and staining ability. In the hedgehog Marshall (104) finds a correlation between maximal interstitial cell development and testicular activity whereas Rasmussen (142) in the woodchuck finds that interstitial cell increase follows considerably behind that of generative tissue activity. When the reproductive portion is again at its minimum state of activity the interstitial cells are maximal. It is seen, therefore, that no definite correspondence is to be found between the interstitial cell activity and the breeding period or the process of gametogenesis. Two hibernating mammals may be entirely opposite in the relationship between increase of Leydig cells and the reproductive activity.

In conclusion one is impressed with the conflicting opinions and lines of evidence relating to interstitial cell activity and the internal secretory function of the testis. The features most impressive to Bouin and Ancel, Steinach and others appear to be open to decided question, and, for many of their opinions there now appears to be a different explanation. Leydig cells in their formation and their increase in testicular changes are apparently ordinary connective tissue cells that have undergone a change (Plato, Bouin and Ancel, Allen, Whitehead, Hofmeister, Kasai,

Sand, Moore, Oslund, Kyrle, Rasmussen, Wagner and others); few modern writers hold the old conception that they are epithelial in origin. When the generative portion suffers a decline the interstitial cells become prominent and when regeneration occurs these appear to return to the ordinary connective tissue state. In other terms, there appears to be somewhat of a balanced relationship between the Leydig cells and the generative portion, perhaps harking back to the nutritional viewpoint initiated by Plato and substantiated more recently by Kyrle. The so-called compensatory hypertrophy appears to be a reaction of the tissues to local or general disturbances in the organ (Moore) rather than having any significance to increased secretory needs of the organism.

When one appreciates the normal quantity and distribution of the Leydig cells in the testes of different mammals, and its variability in the same animal, it becomes difficult to appreciate its significance as a secretory organ. Normally the pig has great quantities of Leydig cells, the horse to a less extent, the cat and others, whereas in the sheep they are scarcely to be found. The dog, most rodents and man have a small quantity in comparison with other mammals. No satisfactory explanation of the secretory need appears to offer a suggestion for these discrepancies.

In general there appears a decline from the pinnacle to which the significance of the interstitial cells has been elevated in the last score of years and at present there are those who strongly proclaim that such cells have absolutely nothing to do with determining or sustaining the secondary sexual characteristics (Brack '22, Cejeka '23 and others). Other tissues of the organ have in turn come in for discussion as the probable source of the internal secretions; the generative portion, some cells of which almost invariably remain after degeneration and appear capable of activity, and the vast Sertoli reticulum, are usually retained in the organ. Discussion of these, however, will not settle the problem and already this account is perhaps too long, though considerably incomplete for an exhaustive discussion of the biology of this organ. Many phases of the tissue reactions as well as the internal secretion question have had to be omitted. It should be clear, however, that the problems pertaining to the mammalian testis are by no means exhausted. Many aspects of both major problems are decidedly open to question, and many particulars await solution but it is certain that the advances of the last half century have been tremendous.

LITERATURE LIST

(1) Albers-Schönberg. 1903. Ueber eine bisher unbekannte Wirkung der Röntgenstrahlen auf den Organismus der Tiere. Münch. Med. Wochenschr., 50: 1859–1860.

(2) Allen, B. M. 1904. The embryonic development of the ovary and testis of the mammals. Amer. Jour. Anat., 3: 89–154.

(3) Allen, Ezra. 1919. Degeneration in the albino rat testis due to a diet deficient in the water soluble vitamine, with a comparison of similar degeneration in rats differently treated and a consideration of the Sertoli tissue. Anat. Record, 16: 93–112.

(4) Ancel and Bouin. 1923. Les cellules séminales ont-elles une action sur les charactères sexuelles? C. R. Soc. Biol., 89: 175–178.

(5) Arlitt, Ada H. and H. G. Wells. 1917. The effect of alcohol on the reproductive tissues. Jour. Exp. Med., 26: 769–778.

(6) Bailey and Miller. 1921. Text-book of Embryology. Wm. Wood and Co. New York.

(7) Bascom, K. F. 1923. The interstitial cells of the gonads of cattle, with especial reference to their embryonic development and significance. Amer. Jour. Anat., 31: 223–259.

(8) Bascom, K. F. 1915. Quantitative studies of the testis. Some observations on the cryptorchid testes of sheep and swine. Anat. Record, 30: 225–241.

(9) Basso, G. L. 1906. Ueber Ovarientransplantation. Arch. f. Gynäk., 77: 51–62.

(10) Belfield, W. T. 1914. Some phases of rejuvenation. Jour. Amer. Med. Assn., 82: 1237–1242.

(11) Benedict and Slack. 1911. A comparative study of temperature fluctuation in different parts of the human body. Carnegie Publications no 155.

(12) Benjamin, H. 1922. The Steinach operation: report of twenty-two cases with endocrine interpretation. Endocrinology 6: 776–786.

(13) Bergoinn, J., and Tribondeau. 1904. Action des rayons X sur les spermatozoides L' Homme. C. R. Soc. Biol., 57: 595–596.

(14) ———. 1904. Action des rayons X sur le testicule du rat blanc. C. R. Soc. Biol., 57: 154–158, 400–402, 592–595.

(15) ———. 1905. Aspermatogenese expérimentale apres une seule exposition aux rayons X. (and other papers). C. R. Soc. Biol., 58: 282–284, 678–680, 1029–1031.

(16) Bertholet, Ed. 1909. Ueber Atrophie des Hodens bei chronischem Alkoholism. Centrl'bl all. Path., 20: 1062–1066.

(17) Berthold. 1849. Transplantation der Hoden. Arch. f. Anat. u. Physiol., p. 42.

(18) Bevan, A. D. 1918. Undescended testes. Surgical clinics of Chicago, 2: 1101–1117.

(19) Bissonnette, T. H. 1924. The development of the reproductive ducts and canals in the free-martin with comparison of the normal. Amer. Jour. Anat. 33: 267–346.

(20) Bland-Sutton, J. 1910. The value of the undescended testis. The Practitioner, 84: 19–30.

(21) Bohn. 1911. Revue Scientifique no. 6.

(22) Bouin and Ancel. 1903. Recherches sur les cellules interstitielles du testicule des mammifères. Arch. de Zool. Exp. et Gen., 4th. ser., 1: 437–522.

(23) ———. 1904. Sur la ligature des canaux déférents chez les animaux jeunes. C. R. Soc. Biol., 56: 84–86.

(24) ———. 1904. Recherches sur la signification physiologique de la glande interstitielle du testicule des mammifères I. Role de la glande interstitielle les individus adultes. Jour. de Physiol., 6: 1012–1022.

(25) ———. 1904. II. Role de la glande interstitielle chez l'embryon, les sujects jeunes et ages; ses variations functionnelles. Jour. de Physiol., 6: 1039–1050.

(26) Bouin and Garnier. 1900. Alterations du tube séminifere au cours de L'alcoholisme expérimental chez le rat blanc. C. R. Soc. Biol., 52: 23–25.

(27) Brack, E. 1911. Ueber innere mannliche genital Missbildungen bei einsitiger Nierenaplasie. Zeitschr. f. Urologie 15. (not seen in original).

(28) ———. 1912. Zur pathologischen Anatomie der Leygigzelle. Virchow's Arch. f. Path. Anat. u. Physiol., 240: 127–145.

(29) Brissaud, E. 1880. Sur les effets de la ligature du canal déférent. Arch. de Physiol., 2nd. ser., 7: 769–789.

(30) Brown, F. T. and A. T. Osgood. 1915. X-rays and sterility. Amer. Jour. Surgery, 18: 179–182 (not seen in original).

(31) Brown-Sequard. 1889. Expérience demontrant la puissance dynamogenique chez L'homme d'un liquide, extrait le testicules d'animaux. Arch. de Physiol., 1 (ser. v): 651.

(32) Busch, August. 1881. Ueber Azoospermie bei gesunden und kranken Menschen nebst einigen Bemerkungen zur pathologischen Histologie des menschlichen Hodens. Zeitschr. f. Biol., 18: 496–521.

(33) Castle, W. E. and J. C. Phillips. 1911. On germinal transplantation in Vertebrates. Carnegie Inst. pub. no. 144.

(34) Cejka, Bohumil. 1923. Eine Studie über die Genese und Funktion des Interstitiums auf Grund der Untersuchungen an senescenten Hoden. Arch. f. entw'mech., 98: 524–578.

(35) Crvolotto, G. 1909. Über verpflanzungen und Gefrierungen der Hoden. Frank. Zeitschr. f. Path., 3: 331–337.

(36) Chapin, Catherine L. 1917. A microscopic study of the reproductive system of foetal free-martins. Jour. Exp. Zool., 23: 453–482.

(37) Corbus, B. C. and V. J. O'Conor. 1922. The familial occurrence of undescended testes. Surgery, Gynecol. and Obstetr., 34: 237–240.

(38) Cordes, H. 1898. Untersuchungen über den Einfluss acuter und chronischer Allgemeinerkrankungen auf die Testikel, speciell auf die Spermatogenese, sowie Beobactungen über das Auftreten von Fett in den Hoden. Virchow's arch., 151: 402–428.

(39) Cooper, Sir Astley. 1830. Observations on the structure and diseases of the testis. London. 1st. ed.

(40) Corner, E. M. 1904. The value of the imperfectly descended testis, the advisability of operation, and the value of the operations performed for its relief. Brit. Med. Jour., 1: 1306–1309.

(41) Crew, F. A. E. 1922. A suggestion as to the cause of the aspermatic condition of the imperfectly descended testis. Jour., Anat. (Lond.), 56: 98–106.

(42) Cunningham. 1917. Text-book of Anatomy. Wm. Wood. New York.

(43) Cunningham, J. T. 1921. Hormones and Heredity. The Macmillan Company. New York.

(44) Davis, D. M. 1924. Pathological anatomy and histology of the testicle. Endocrinology and metabolism. Appleton. New York, 2: 473–490.

(45) Felizet, G., and A. Branca. 1898. Histologie du testicule ectopique. Jour. de Anat. et Physiol., 34: 589–641.

(46) ———. 1902. Recherches sur le testicule en ecotopie. Jour. de Anat. et Physiol., 38: 329–442.

(47) Foa, C. 1901. Sur la transplantation des testicules. Arch. Ital. de Biol., 35: 337–348.

(48) Foges, A. 1898. Zur Hodentransplantation bei Hahnen. Centrl'bl f. Physiol., 12: 898–901.

(49) Frieben. 1903. Hodenveranderung bei Tieren nach Rontgenbestrahlungen. Münch. Med. Wochenschr., 50: 2295.

(50) Fukui, N. 1923. On a hitherto unknown action of heat ray on testicles. Japan Medi-Medical World, 3.

(51) ———. 1923. Action of body temperature on the testicle. Japan Medical World, 3.

(52) ———. 1923. On the action of heat rays upon the testicle: An histological, hygienic and endocrinological study. Acta Scholae Med. Univ. Imp. in Kito., 6 (fasc. 11): 225–258.

(52A) Gantini, C. 1903. Les cellules interstitielles du testicule chez les animaux hibernants. Arch. Ital. de Biol., 40: 323–324.

(53) Gobell, R. 1898. Versuche über Transplantation des Hodens in die Bauchhole. Centrl' bl. f. All. Path. Anat., 9: 737–739.

(54) Godard, M. E. 1856. Études sur la monorchidie et la cryptorchidie. C. R. Soc. Biol., 2nd ser., 3: 315–460.

(55) Goddard, T. R. 1920. Hypertrophy of interstitial tissue of the testis in man. Jour. Anat. (Lond.), 54: 173–176.

(56) Gosselin, L. 1847. Memoire sur les obliterations de voies spermatique. Arch. Gen. de Med., 4th. Ser. 14: 405–424, 15: 40–52.

(57) Goubaux, A. and E. Follin. 1855. De la cryptorchidie chez l'homme et les prinapaux animaux domestiques. C. R. Soc. Biol., 2nd. ser., 2: 293–330.

(58) Griffiths, Joseph. 1893. The structural changes in the testicle of the dog when it is replaced within the abdominal cavity. Jour. Anat. and Physiol. (Lond.), 27: 482–499.

(59) ———. 1894. Retained testes in man and in the dog. Jour. Anat. and Physiol. (Lond.), 28: 209–220.

(60) ———. 1894. The condition of the testes and prostate gland in eunuchoid persons. Jour. Anat. and Physiol. (Lond.), 28: 221–224.

(61) ———. 1895. Three lectures on the testis. The Lancet, 1: 791–798, 916–920.

(62) ———. 1896. The effects upon the testis of ligature of the spermatic artery, spermatic veins, and of both artery and veins. Jour. Anat. and Physiol. (Lond.), 30: 81–105.

(63) Haire, Norman. 1925. Rejuvenation. The MacMillan Company. New York.

(64) Hanau, A. 1897. Versuche über den Einfluss der Geschlechtsdrusen auf die Secundaren Sexualcharactere. Arch. f. d. Gesammte Physiol., 65: 516–517.

(65) Hanes, F. M. 1911. Relation of interstitial cells of Leydig to the production of an internal secretion by the mammalian testis. Jour. Exp. Med., 13: 338–354.

(66) Hanes, F. M. and J. Rosenbloom. 1911. A histological and chemical study of the fatty matter of normal and cryptorchid testes. Jour. Exp. Med., 13: 355–364.

(67) Harvey, W. G. 1908. On the pathological effects of Roentgen rays on animal tissues. Jour. of Pathology, 12: 549–556.

(68) Hansemann, D. 1895. Ueber die sogenannten Zwischenzellen des Hodens und deren Bedeutung bei pathologischen veranderungen. Virchow's Arch., 142: 538–546.

(69) Hatai, S. 1915. The growth of organs in the albino rat as affected by gonadectomy. Jour. Exp. Zool., 18: 1–45.

(70) Herlitzka, A. 1900. Recherches sur la transplantation, La transplantation des ovaries. Arch. Ital. de Biol., 34: 84–118.

(71) Herxheimer, G., and K. F. Hoffmann. 1908. Ueber die anatomischen Wirkungen der Rontgenstralen auf den Hoden. Deutsch. Med. Wochenschr., 34: 1551.

(72) Hewer, Evelyn E. 1916. The structure of the thymus gland and of the reproductive organs in white rats, together with some observations on the breeding capacity of these animals. Jour. Physiol. (Lond.), 50: 434–458.

(73) Hofmeister, F. 1872. Untersuchungen über die Zwischensubstanz im Hoden der Saugethiere. Sitz' ber. d. Kais. Ak. d. Wissensch., 65 (Abt. 3): 77–100.

(74) Hunt, H. L. 1922. Experiences in testicle transplantation. Endocrinology, 6: 652–654.

(75) Hunter, John. 1880. Observations on Certain Parts of the Animal Economy. Philadelphia. Haswell, Barrington and Haswell, edition 1880.

(76) Jacobson, A. 1879. Zur pathologischen Histologie der traumatischen Hodenentzundung. Arch. f. Path. Anat. u. Physiol. 75: 349–397.

(77) Kabai, K. 1908. Über die Zwischenzellen des Hodens. Virchow's Arch. f. Path. Anat. u. Physiol., 194: 1–17.

(78) Keyes, E. J., and D. W. MacKenzie. 1917. The operative treatment of cryptorchidism. Jour. Amer. Med. Assoc., 68: 349–351.

(79) Kostitch, A. 1921. Sur L'involution du processus spermatogenetique provoquee par L'alcoholisme Expérimental. C. R. Soc. Biol., 84: 674–676.

(80) Kuntz, Albert. 1919. Experimental degeneration in the testis of the dog. Anat. Record, 17: 211–233.

(81) ———. 1921. Degenerative changes in the seminal epithelium and associated hyperplasia of the interstitial tissue in the mammalian testis. Endocrinology, 5: 190–204.

(82) Kyrle, J. 1911. Über die regenerationsvorgange im tierischen und menschlichen Hoden. Sitz'ber. d. Kais. Akad. Wien. 120 (Abt. 3): 1–124.

(83) Lenhossek (von) M. 1897. Beitrage zur Kenntniss der Zwischenzellen des Hodens. Arch. f. Anat. u. Physiol., pp. 65–85.

(84) Lespinnasse, V. D. 1913. Transplantation of the testicle. Jour. Amer. Med. Assn., 61: 1869.

(85) ———. 1914. The testicle as a gland of internal secretion. Endocrinology and Metabolism (Appleton, N. Y.), 2: 491–522.

(86) Leydig, F. 1850. Zur Anatomie der mannlichen Geschlechtsorgane und Analdrusen der Saugethiere. Zeitschr. f. Wissensch. Zool., 2: 1–57.

(87) Lichtenstern, Robert. 1914. Die Überpflanzung der mannlichen Keimdruse. Wien. Julius Springer.

(88) Lillie, F. R. 1917. The free-martin: a study of the actions of sex hormones in the foetal life of cattle. Jour. Exp. Zool., 23: 371–452.

(89). ———. 1923. Supplementary notes on twins in cattle. Biol. Bulletin, 44: 47–78.

(90) Lillie, F. R., and K. F. Bascom. 1922. An early stage of the free-martin and the parallel history of the interstitial cells. Science, 55: 624–625.

(91) Lipschutz, A. 1919. Die Pubertatsdruse und ihre Wirkungen. Berne.

(92) ———. 1922. The socalled compensatory hypertrophy of the testicle after unilateral castration. Jour. Physiol. (Lond.), 56: 451–458.

(93) ———. 1924. The internal secretions of the sex glands. Baltimore, Williams & Wilkins Co.

(94) Lipschutz, Ottow, and Wagner. 1921. Du pole inférior du testicule dans la castration partielle (and other short papers). C. R. Soc. Biol., 83: 140; 85: 42, 86, 88, 638.

(95) ———. 1922. On the hypertrophy of the interstitial cells in the testicle of the guinea pig under different experimental conditions. Proc. Roy. Soc., B., 93: 132–142.

(96) Lode, A. 1895. Zur Transplantation der Hoden bei Hahnen. Wien. Klinische Wochenschr., 8: 345.

(97) Lono, E. R. 1924. Tuberculosis reinfection and the tuberculin reaction in the testicle of the tuberculous guinea pig. Amer. Jour. of Tuberculosis, 9: 215–253.

(98) Lono, E. R., and M. Seyfarth. 1924. The testicle as an indicator of allergy in the hypersensitiveness of infection and anaphalaxis. Amer. Jour. of Tuberculosis, 9: 254–263.

(99) Lubarsch, O. 1896. Ueber das Vorkommen krystallinischer und krystalloider Bildungen in den Zellen des menschlichen Hodens. Arch. f. Path. Anat. u. Physiol., 145: 316–335.

(100) Lydston, G. F. 1916. Sex gland implantation. Jour. Amer. Med. Assn., 66: 1540–1543.

(101) ———. 1918. Cases showing remote results of testicle implantation. Jour. Amer. Med. Assn., 70: 907–908..

(102) ———. 1919. Further observations on sex gland implantation. Jour. Amer. Med. Assn., 72: 396–398.

(103) McKenna, C. M. 1921. Testicle transplantation. Report and demonstration of a case. Illinois Med. Jour., 40: 228–229.

(104) Marshall, F. H. A. 1911. The male generative cycle in the hedgehog. Jour. of Physiol. (Lond.), 43: 247–259.

(105) ———. 1912. On the effects of castration and ovariotomy upon sheep. Proc. Roy. Soc. (Lond.), B., 85: 27.

(106) Marshall and Jolly. 1907. Results of removal and transplantation of ovaries. Trans. Roy. Soc. Edinburgh, 45.

(107) Mason, K. E. 1925. A histological study of sterility in the albino rat due to a dietary deficiency. Proc. Nat. Acad. Sci., 11: 377–382.

(108) Mattill, H. A., and J. S. Carman. 1923. The degeneration of the testis of rats on a milk diet. Proc. Soc. Exp. Biol. and Med., 20: 420.

(109) Maximow, A. 1899. Die histologischen Vorgange bei der Heilung von Hodenverletzungen und die Regenerationsfahigkeit des Hodengewebes. Beitrage z. Path. Anat., 26: 230–316.

(110) Mills, R. G. 1919. The pathological changes in the testes in epidemic pneumonia. Jour. Exp. Med., 30: 505–529.

(111) Monod, C., and G. Arthaud. 1887. Contribution a l'étude des alterations du testicule ectopique et de leurs consequences (infencondite). Arch. gen. de Med. 7th ser. 20: 641–652.

(112) Moore, Carl R. 1919. On the properties of the gonads as controllers of somatic and psychical characteristics: 1. The Rat. Jour. Exp. Zool., 28: 137–160.

(113) ———. 1919. II. Growth of gonadectomized male and female rats. Jour. Exp. Zool., 28: 459–467.

(114) ———. 1920. The production of artificial hermaphrodites in mammals. Science, 52: 179–182.

(115) ———. 1921. III. Artificial hermaphroditism in rats. Jour. Exp. Zool., 33: 129–171.

(116). ———. 1921. IV. Gonad transplantation in guinea pig. Jour. Exp. Zool., 33: 365–389.

(117) ———. 1922. V. The effects of gonadectomy in the guinea pig, on growth, bone length, and weight of organs of internal secretion. Biol. Bull., 43: 285–312.

(118) ———. 1922. Cryptorchidism experimentally produced. Proc. Soc. Zool. Anat. Rec., 24: 383.

(119) ———. 1923. On the relationship of the germinal epithelium to the position of the testis. Proc. Assoc. Anat. Anat. Rec., 25: 142.

(120) ———. 1924. The behavior of the germinal epithelium in testis grafts and in experimental cryptorchid testes (rat and guinea pig). Science, 59: 41–44.

(121) ———. 1924. The behavior of the testis in transplantation, experimental cryptorchidism, vasectomy, scrotal insulation, and heat application. Endocrinology, 8: 493–508.

(122) ———. 1924. VI. Testicular reactions in experimental cryptorchidism. Amer. Jour. Anat., 34: 269–316.

(123) ———. 1924. VIII. Heat application and testicular degeneration; the function of the scrotum. Amer. Jour. Anat., 34: 337–358.

(124) ———. 1925. Sex determination and sex differentiation in birds and mammals. The Amer. Naturalist, 59: 177–190.

(125) ———. 1926. IX. Testis graft reactions in different environments (rat). Amer. Jour. Anat., Vol. (in press).

(116) Moore, Carl R., and H. D. Chase. 1923. Heat application and testicular degeneration. Proc. Soc. Zool. Anat. Rec., 26: 344.

(127) Moore, Carl R., and Wm. J. Quick. 1923. A comparison of scrotal and peritoneal temperatures. Proc. Soc. Zool. Anat. Rec., 26: 344.

(128) ———. 1924. The scrotum as a temperature regulator for the testes. Amer. Jour. Physiol., 68: 70–79.

(129) ———. 1924. VII. Vasectomy in the rabbit. Amer. Jour. Anat., 34: 317–336.

(130) Moore, Carl R., and R. Oslund. 1923. Experimental studies on sheep testes. Proc. Soc. Zool. Anat. Rec., 26: 343.

(131) ———. 1924. Experiments on the sheep testis-cryptorchidism, vasectomy and scrotal insulation. Amer. Jour. Physiol., 67: 595–607.

(132) Morris, R. T. 1916. A case of testicle grafting with unexpected results. Jour. Amer. Med. Assn., 67: 741–742.

(133) Myers, B. D. 1915. Histological changes in testes following vasectomy. Proc. Soc. Anat. Anat. Record, 10: 228.

(134) Oslund, Robt. 1924. A study of vasectomy on rats and guinea pigs. Amer. Jour. Physiol., 67: 422–443.

(135) ———. 1924. Vasectomy on dogs. Amer. Jour. Physiol., 70: 111–117.

(136) ———. 1924. Interstitial cell hypertrophy. Amer. Jour. Physiol., 69: 589–598.

(137) Oslund, Robt. 1914. Vasectomy and rejuvenescence. Science, 60: 419–421.

(138) Philipp, 1907. Effects of X-rays on testes in man. Quoted in Osler's Modern Medicine, Vol. 1, p. 61.

(139) Plato, J. 1897. Die interstitiellen Zellen des Hodens und ihre physiologische Bedeutung. Arch. f. Mik. Anat., 48: 250–304.

(140) ———. 1897. Zur Kenntniss der Anatomie und Physiologie des Geschlechtsorgane. Arch. f. Mik. Anat., 50: 640–685.

(141) Poncet, A. 1903. De l'influence de la castration sur le dévelopment du squellette. C. R. Soc. Biol., 55: 65–67.

(142) Rasmussen, A. T. 1917. Seasonal changes in the woodchuck (marmota monax). Amer. Jour. Anat., 22: 475–515.

(143) ———. 1918. Cyclic changes in the interstitial cells of the ovary and testis in the woodchuck (marmota monax). Endocrinology, 2: 353–404.

(144) Regaud, C. 1904. État des cellules interstitielles du testicule chez la taupe pendant la periode de spermatogenese et pendant l'état de repos de canalicules séminaux. C. R. d. l'assoc. d. Anat. p. 54 (not seen in original).

(145) Regaud and J. Blanc. 1906. Action des Rayons X sur les diverses generations de la lignee spermatique. Extreme sensibilite des spermatogonies a ces rayons (and other titles). C. R. Soc. Biol., 61: 163–165, 390–392, 652–654, 731–733.

(146) Regaud and G. Dubreuil. 1907. Action des rayons de Rontgen sur le testicule de lapin. I. conservation de la puissance virile et sterilization. C. R. Soc. Biol., 63: 647–649.

(147). ———. 1908. Action res rayons de Rontgen sur le testicule des animaux impubères; immunite (relative) de l'epithelium séminale. C. R. Soc. Biol., 65: 393–395.

(148) Regaud and Th. Nogier. 1909. Sterilization complete et definitive des testicules du rat, sans acune lesion de la peau, par une application unique de rayons X filtres. C. R. Acad. Sci., 149: 1398–1401.

(149) Regaud and Policard. 1901. Étude comparative du testicule du porc normal, impubère et ectopique, au point de vue des cellules interstitielles. C. R. Soc. Biol., 53: 450–452.

(150) Reinke. 1896. Beitrage zur Histologie des Menschen. Arch. f. Mik. Anat., 47: 34–44.

(151) Retterer, Ed. 1919. Evolution des greffes testiculaires sur le bouc. C. R. Soc. Biol., 82: 1022–1025.

(152) Retterer, Ed. 1919. Evolution des greffes testiculaires du belier. C. R. Soc. Biol., 82: 1099–1102.

(153) Retterer, and S. Voronoff. 1921. Evolution du testicule apres ligature des vaisseux testicularies. C. R. Soc. Biol., 85: 153–156.

(154) ———. 1922. Effets locaux et généraux dus a la resection des canaux déférents. C. R. Soc. Biol., 86: 1073–1075.

(155) Ribbert, Hugo. 1898. Über Transplantation von Ovarium, Hoden und Mamma. Arch. f. Entw'mech., 78: 688–708.

(156) Richon, M. L., and P. Jeandelize. 1903. Influence de la castration et de la resection sur le dévelopment des organes genitau externes chez le jeune lapin. Role des cellules interstitielles du testicule. Hypothese sur la pathogenie de l'infantilisme. C. R. Soc. de Biol., 55: 1685–1687.

(157) Sand, Knud. 1919. Experiments on the internal scretion of the sexual glands, especially on experimental hermaphroditism. Jour. Physiol. (Lond.), 53: 257.

(158) ———. 1921. Etudes expérimentales sur les glandes sexuelles chez les mammifères. Jour. de Physiol., 19: 304–322.

(159) ———. 1921. Expériences sur la resection du "vas deferens". Jour. de Physiol., 19: 494–503.

(160) ———. 1921. Etudes experimentales sur les glandes sexuelles chez les mammiferes. Cryptorchidie experimentale. Jour. de Physiol., 19: 515–527.

(161) ———. 1921. Vasectomie pratiquee chez ein chien dans un but de regeneration. C. R. Soc. Biol., 85: 1201.

(162) ———. 1922. L'Hermaphrodisme expérimental. Jour. de Physiol., 20: 472–487.

(163) ———. 1923. Vasoligature (epididymectomy) employed ad mod. Steinach with a view to restitution in cases of senium and other states (impotency, depression). Acta Chirurgica Scandinavica, 55: (fasc. 4) 387–416.

(164) ———. 1923. Experiments on the endocrinology of the sexual glands. Endocrinology, 7: 273–301.

(165) Schultz. 1900. Transplantation der Ovarien auf Mannliche thiere. Centrl'bl. f. all. path., 2: 200.

(166) Schmidt, Peter. 1924. The Theory and Practice of the Steinach Operation. London. Wm. Heinemann.

(167) Sellheim. 1899 (abstract). Jahresbericht der Anat., 5: I., p. 191, II., p. 121.

(168) Seyfarth, MacHarper. 1924. The abortin

reaction in the testicle as an indicator of the hypersensitiveness of infection. Jour. Infectious Diseases, 35: 489–497.

(169) Shattock, S. G., and C. G. Seligmann. 1904. Observations upon the acquirement of secondary sexual characters, indicating the formation of an internal secretion by the testicle. Proc. Roy. Soc. Lond., 73: 49 and 1905-Trans. Path. soc. Lond., 56: 57–80.

(170) Simmonds. 1898. Ueber die Ursachen der Azoospermie. Berl. Klin. Wochenschr., 35: 806.

(171) ———. 1913. Ueber Mesothoriumschadigung des Hodens. Deut. Med. Wochenschr., 39: 2291–2292.

(172) ———. 1921. Ueber das Verhalten des menschlichen Hodens bei narbigen Samenleiterverschluss. Deut. Med. Wochenschr., 47: 665.

(173) Spencer, Herbert. 1899. The Principles of Biology. Appleton. New York.

(174) Stanley, L. L. 1921. Testicular substance implantation. Endocrinology, 5: 708–714.

(175) ———. 1922. An analysis of one thousand testicular substance implantations. Endocrinology, 6: 787–794.

(176) Stanley and G. D. Kelker. 1920. Testicle transplantation. Jour. Amer. Med. Assn. 75: 1501–1503.

(177) Steinach, E. 1910. Geschlechtstrieb und echt sekundare Geschlechtsmerkmale als Folge der innersekretorischen Funktion der Keimdrusen. Centrl'bl. f. Physiol., 24: 551–566.

(178) ———. 1911. Umstimmung des Geschlechtscharakters bei Saugetieren durch Austausch der Pubertatsdrusen. Centrl'bl. f. Physiol., 25: 723–725.

(179) ———. 1912. Willkurliche Umwandlung von Saugetieremannchen in Tiere mit ausgepragt Weiblichen Geschlechtscharakteren und Weiblicher Psyche. Pflugers Archiv., 144: 71–106.

(180) ———. 1913. Feminierung von Mannchen und Maskulierung von Weibchen. Centrl'bl. f. Physiol., 27: 717–723.

(181) ———. 1916. Pubertatsdrusen und Zwitterbildung. Arch. f. Entw'mech., 42: 307–330.

(182) ———. 1920. Kunstliche und naturliche Zwitterdrusen und ihre analogen Wirkungen. Arch. f. Entw'mech., 46: 12–28.

(183) ———. 1920. Verjungung durch experimentells Neubelebung der alternden Pubertatsdruse. Arch. f. Entw'mech., 46: 557–618.

(184) Steinach and Holzknecht. 1916. Erhohte Wirkungen der inner Sekretion bei Hypertrophie der Pubertatsdrusen. Arch. f. Entwmech., 42: 490–505.

(185) Stieda, L. 1897. Die Leydig'schen Zwischensubstanz des Hodens. Arch. f. Mik. Anat., 48: 692–695.

(186) Tandler, J., and S. Grosz. 1907. Ueber den Einfluss der Kastration auf den Organismus. Wien. Klin. Wochenschr., 20: 1596.

(187) ———. 1908. Untersuchungen an Skopzen. Wien. Klin. Wochenschr., 21: 277–282.

(188) ———. 1910. Über den Einfluss der Kastration auf den Organismus. Arch. f. Entwmech., 30: 236–253.

(189) ———. 1911. Über den Saisondimorphismus des Maulwurfhodens. Arch. f. Entwmech., 33: 297–302.

(190) Tiedje, H. 1921. Unterbindungsbefunde am Hoden unter Berucksichtigung der Pubertatsdrusenfrage. Deut. Med. Wochenschr., 47: 352–354.

(191) Thorek, M. 1922. The present position of testicle transplantation in surgical practice; a preliminary report of a new method. Endocrinology, 6: 771–775.

(192) ———. 1924. The human testis and its diseases. Lippincott. Philadelphia.

(193) van Wagenen, G. 1924. Degeneration of the germinal epithelium in the testis of the rat as a result of efferent duct ligation. Proc. Soc. Anat. Anat. Record, 27: 189.

(194) ———. 1925. Changes in the testis of the rat following ligation of the ductuli efferenti. Proc. Soc. Anat. Anat. Record, 29: 399.

(195) Villemin, F. 1906. Sur la regeneration de la glande seminale après destruction par les rayons X. C. R. Soc. Biol., 60: 1076–1077.

(196) Voronoff, S. 1923. Greffes Testiculaires. Paris. Doin.

(197) Wagner, Karl. 1925. Sind die Zwischenzellen des Saugetierhodens Drusenzellen? Biologia Generalis, 1: 22–51.

(198) Wallace, C. S. 1905. The results of castration and vasectomy upon the prostate gland in the enlarged and normal condition. Trans. Path. Soc. (Lond.), 56: 80–106.

(199) Wakelin Barratt, J. O. and G. Arnold. 1911. Cell changes in the testis due to X-rays. Arch. f. Zellforschung, 7: 242–276.

(200) Weichselbaum A., and J. Kyrle. 1912. Über die Veranderung der Hoden bei chronischem Alkoholismus. Sitz'ber. d. Kais. Akad. Wien., 121: 51–70.

(201) Wheelon, C. H. 1911. Observations on two testes nine months following vasectomy. Endocrinology, 5: 307–308.

(202) Whitehead, R. H. 1904. The embryonic development of the interstitial cells of Leydig. Amer. Jour. Anat., 3: 167–182.

(203) ———. 1904. Studies of the interstitial cells of Leydig. 2. Their post embryonic development. Amer. Jour. Anat., 4: 193–197.

(204) ———. 1908. Studies of the interstitial cells of Leydig. 3. Histology. Anat. Record, 1: 213–227.

(205) ———. 1908. A peculiar case of cryptorchidism and its bearing upon the problem of the function of the interstitial cells of the testis. Anat. Record, 2: 177.

(206) ———. 1909. The interstitial cells of an hermaphrodite horse. Anat., Record, 3: 264.

(207) ———. 1911. On the chemical nature of certain granules in the interstitial cells of the testis. Amer. Jour. Anat., 14: 63–71.

(208) ———. 1913. The structure of a testis from a case of human hermaphroditism. Anat. Record, 7: 83–90.

(209) Wiedersheim. 1907. Comparative Anatomy of Vertebrates. The Macmillan Company. New York.

(210) Willier, B. H. 1911. The structures and homologies of the free-martin gonads. Jour. Exp. Zool., 33: 63–127.

(211) Wolbach, S. B. 1919. Comments on the pathology of fatal influenza cases, as observed at Camp Devens, Mass. Johns Hopkins Hospital Bull., 30: 104–109.

(212) Woodland, W. 1903. On the phylogenetic cause of the transposition of the testes in mammalia; with remarks on the evolution of the diaphragm and the metanephric kidney. Proc. Zool. Soc. Lond., 1903, part 1.: 319–340.

SYMBIOSIS AMONG ANIMALS WITH SPECIAL REFERENCE TO TERMITES AND THEIR INTESTINAL FLAGELLATES

By L. R. CLEVELAND

Department of Tropical Medicine, Harvard University Medical School, Boston, Massachusetts

1. INTRODUCTION

Origin and meaning of term symbiosis

THE term symbiosis as originally used by De Bary (1879) means the living-together of two organisms on a partnership basis. The partnership may be between animal and plant, animal and animal, plant and plant. Such associations vary vastly in degree, but each organism must receive some benefit from its partner or else the association is not one of symbiosis. It is also usually true that one partner is much smaller than the other, the small one being the symbiote or parasite and the large one the host, which, strictly speaking, is also a symbiote or partner. Host and parasite are parasitological terms for the symbiotes or partners. Meyer (1925) has recently pointed out that the term symbiont is a misnomer; the Greek word for "companion" or "partner" is symbiote.

How symbiosis is studied

It is a most difficult matter, indeed, to tell where symbiotic associations end and others begin. The only crucial method available in most cases at present is to first break the association, study each partner separately, determine what it can and cannot do when alone, then reestablish the partnership and study the reunited partners. Such a procedure is infinitely more difficult than to reason how one partner, particularly the microscopic one, might easily help the other; consequently very few definitely established examples of symbiosis are known. Most of our information consists of possibilities.

Some examples of symbiosis

The intracellular and intestinal yeast-like and bacteria-like bodies present in all individuals of many groups of insects, and transmitted in most cases from generation to generation through the egg, may or may not be symbiotes—nobody knows. The literature on the presence, character, and possible symbiotic rôle that these microörganisms play in the lives of their insect hosts is immense (Portier, 1918; Buchner, 1921; Uichanco, 1924; Meyer, 1925; give extensive references), and it is unnecessary to add to it by giving a discussion here. What needs to be done is to devise some method or combination of methods by which these microörganisms may all be taken from their hosts without injury—a difficult undertaking. Here is a most fruitful field of investigation awaiting an opening. Who will open it?

The intracellular bacteria of certain mollusks have been termed symbiotes, although Meyer (1925), who has recently made a detailed investigation of some of these microörganisms in relation to their molluskan hosts, says the word symbiote

"is used more for the sake of convenience than as the result of an absolute conviction that the phenomenon is one of true symbiosis." He continues, "from the experiments and findings reported it is easy to assume that the mollusks derive some benefit from the intracellular bacteria as anabolists or catabolists of metabolic waste products, but, what possible benefit can the microörganisms derive from the association?"

Green (*Zoochlorella*) and yellow (*Zooxanthella*) algae are closely associated with many animals: Turbellarians, Mollusks, Annelids, Bryozoans, Coelenterates, Rotifers, and Protozoa. In some of these animal-plant associations it has been claimed by a number of investigators that the gas liberated contains much more than the normal percentage of oxygen, in some associations as high as 55 per cent; whereas the colorless varieties of these animals, that is those without algae, give off a very much smaller percentage of oxygen, if any at all. Such associations are regarded as helpful or beneficial to both partners. The algae supply the animal with oxygen for which they get carbon dioxide and nitrogen in return. Perhaps a fair number of these associations are beneficial to both partners, but certainly very few of them are absolutely necessary for the existence of the partners concerned. In one of the Turbellaria, *Convoluta roscoffensis*, the larvae, according to Keeble and Gamble, are colorless, that is, do not harbor algae, at the time of hatching and can only feed themselves for a week, by which time they have gotten algae. For a while, then, they live on the products given off by the algae, but later, after having reached a more advanced stage in their life-cycle, they become capable of digesting the algae, and the symbiosis—at least for the algae digested— ends and the association merges into true parasitism.

Minute bacteria-like luminescent bodies associated with fishes, tunicates, cephalopods, and insects have been studied by a number of investigators, and it is practically certain that at least some of these associations are symbiotic in character, that is to say, each of the organisms is benefited in some way, however slight the benefit may be.

Relation of symbiosis to other forms of parasitism

One of the most interesting things about symbiosis is its origin and relation to other host-parasite associations: commensalism, that association in which hosts tolerate their parasites as guests or messmates; and true parasitism, that association in which the parasites live altogether or in part at the expense of their hosts. In a commensalistic association neither party is benefited nor injured. Obviously, associations with such an equilibrium must be extremely rare, for many of the so-called commensalistic parasites cannot live outside of their hosts and, of course, must be benefited. In true parasitism either the host or the parasite is injured: when a host develops immunity and frees itself of parasites, the parasites are injured; until this occurs the host receives the principal injury. Parasitism proper, or in the general sense, according to fairly well-established usage, covers all three associations: commensalism, symbiosis, and true parasitism. It would be much better, however, to use the term symbiosis, which means living together, and under it include commensalism, mutualism, and parasitism. However desirable such a change in terminology may be, it is not an easy matter to make it, and I shall not attempt it at this time.

Symbiosis occupies perhaps somewhat of a mid-way position between commensalism and true parasitism, and, from our meagre information concerning its possible

origin, is probably derived from them—just how, we do not know. Ideal partnership or symbiosis, that condition in which each party concerned gives as much as it receives, is rarely, if ever, realized; the partnership is usually one-sided, that is, one partner does most of the giving. Such associations from the standpoint of the partner that does most of the giving, are near true parasitism; from the standpoint of the partner that does most of the receiving, they are near commensalism.

II. GENERAL CONSIDERATIONS OF TERMITES AND THEIR PROTOZOA

Species and families harboring protozoa

Approximately 1500 species of termites have been described and have been grouped in four families: Mastotermitidae, Kalotermitidae, Rhinotermitidae, and Termitidae. All species that have been examined from the first three families (Cleveland, 1923) have been found to harbor a teeming menagerie of intestinal protozoa—the protozoa in these insects weigh almost as much as the insects themselves. Most species of the Termitidae that have been examined have not been found to harbor any protozoa at all, and in those where they have been found the quantity can in no way be compared to that of the other families. Dr. Harold Kirby has recently collected and shipped me a large number of termites from Panama and Costa Rica. Small amoebae and flagellates are present, but not abundant, in two species of *Amitermes*, *A. beaumonti* Banks and *H. medius* Banks. Larger, more abundant and wood-ingesting amoebae are present in *Mirotermes panamaensis* Snyder. Small tetramitid flagellates are present in *Orthognathotermes wheeleri* Snyder. Most of the Termitidae examined had no protozoa. A list of the Termitidae examined and a description of the protozoa found, will be published by Dr. Kirby soon.

More investigation may determine whether or not the Termitidae are losing their protozoa or are just beginning to harbor them. The situation is a most interesting one, indeed, and certainly should be investigated thoroughly. Are we observing in this family of termites the beginning or the last stage in the closing of a symbiotic association?

Partnerships with fungi

It has been shown by Döflein (1905), Petch (1906), Fuller (1920, 1921) and others that some of the Termitidae with no protozoa in them have formed partnerships with fungi by giving over a certain portion of their nest (the fungus garden) to the growth and cultivation of the fungus which they eat along with soil, cellulose, and hemicellulose which the fungus has acted upon. Fuller (1921) has observed the deliberate removal of certain parts of the fungus garden during prolonged droughts followed by a week or so of rain to propitious places outside the nest.

Food

The Termitidae as a rule do not feed solely on wood or cellulose material, and when they do eat wood, it is usually very much more decayed than that which the protozoa-harboring families eat. The Kalotermitidae feed on wood that has begun to decay very little if at all—several species feed on hard, dry wood that has not begun to decay in the least. Two genera of the Rhinotermitidae (*Reticulitermes* and *Termopsis*) have lived for more than two years on a diet of pure cellulose (Cleveland, 1925a). A number of efforts have been made recently to get Termitidae to live on a cellulose (filter paper) diet. *Obtusitermes*, *Anoplotermes*, *Orthognathotermes*, *Mirotermes*, and *Amitermes* in large numbers have been given a cellulose diet

under various conditions as near natural as was possible in the laboratory. Some of them ate the cellulose, but others did not, and none of them were able to live longer than a month. They usually died much sooner. However, it is a difficult matter to keep most Termitidae in confinement, and for this reason it should not be stated yet that these termites are unable to live on a cellulose diet. It is only after considerable experience, in fact, that one is able to keep any termite perfectly normal under laboratory confinement. If these termites are able to live on a cellulose diet, it is highly probable that they are assisted in some way by microörganisms. It is not likely that they digest cellulose themselves, although we do not know of any symbiotes that do it for them. Many of them are filled with a wriggling horde of large spirochaetes, which may be easily removed and without injury to their host by confining the termites in oxygen under pressure for about the same time that it takes to remove or kill termite protozoa. Some of them have intestinal fungi, but we do not know what rôle these fungi and spirochaetes play in their host's metabolism. This should be determined.

Termite protozoa

The protozoa of termites are most interesting creatures indeed. Everyone who has observed them has marvelled at their abundance, interesting structures, peculiar adaptation and mode of living, and has wondered how on earth so many of them live in a single termite and what they do, that is, how they live and what relation they have to the termites in which they live, move, and have their being.

It has been stated by a number of investigators that protozoa are present only in the workers and soldiers. This is not true. They are present in all wood-eating and wood-feeding members of a colony. In those termites where a worker caste is present, the old and enlarged reproductive adults do not feed on wood—the workers feed them, and when they do, the protozoa disappear. When no worker caste is present, the wood-feeding habit is never given up, the protozoa are never lost, and no greatly enlarged adults are found.

As a rule protozoa—living or dead—are not present in the fecal pellets of termites. They do not pass out with fecal material in the way that the intestinal protozoa of other animals usually do. They are perhaps used as food for their host. Young termites, however, seem to get protozoa, in some unknown manner, from the ani of the older members of the colony. Some special provision seems to be made to give them to the young, perhaps by giving off extra and more liquid material than pellets, from the anus. It is remarkable how soon the larvae get protozoa in their guts. Out of twenty-five larvae of *Termopsis* experimented with, fifteen acquired protozoa within twenty-four hours from the time of hatching. Larvae kept by themselves from the time of hatching never get protozoa and they die in a week or thereabouts.

The plates at the end of this paper show several genera of termite protozoa selected at random from more than forty that are known. Evidently only a beginning has been made in the study of these protozoa; one can find new genera and species in almost any termite. Seven or eight genera sometimes occur in a single termite host; in fact it is most unusual to find less than four genera, except in the Termitidae. It is quite probable that when all the termite-inhabiting protozoa are known, representatives of practically all parasitic or entozoic protozoa of other animals will be found in termites. Does this mean that the evolution of parasitic protozoa has

been going on longer in termites than in other animals, or does it mean that protozoa have found a much more favorable environment in termites than in other animals? Does a symbiotic association, which should give the most favorable environment for a protozoon, encourage evolution? Do more changes occur, or are more of those that do occur preserved? We do not know. What little information we have regarding the geographical distribution of termites and their intestinal protozoa indicates that there has been a parallel evolution of the two groups of organisms, which, if true, means that the symbiosis has perhaps been established for a very long time. It is also probably true that many pathogenic protozoa have become extinct by eliminating their hosts, while symbiotic protozoa have made it possible for many variations in their hosts to survive and for their hosts to become very numerous; and if commensalistic protozoa were once pathogenic, there has perhaps been a great loss of species and genera in their evolution—only the less pathogenic ones survived and gradually became commensals.

III. METHODS AND RESULTS OF FREEING TERMITES OF THEIR PROTOZOA

Incubation

A fairly convenient method of freeing termites of their protozoa is to incubate them at 36°C. for twenty-four hours (Cleveland, 1924). Such a treatment perhaps injures some of them but not a great deal when the proper moisture concentration is maintained. This perhaps can best be done by confining the termites with wood in a vaseline-sealed chamber with a very small amount of moist cotton. With no moisture, the termites become too dry and are injured; with a great deal of moisture, molds grow rapidly and injure them.

When the common eastern termite, *Reticulitermes flavipes* Kollar, is incubated as described above, all of its intestinal protozoa are removed within twenty-four hours; all protozoa in most individuals are killed in twenty hours. The differential death points of termites and their protozoa when incubated or heated for ten minutes are great enough to allow the removal of the protozoa without killing the termites, but such a method has not proven as useful, so far, as one with a longer heating period.

Experiments have been carried out recently on the incubation of many species of termites from Panama and Costa Rica, the details of which, when completed, will appear in another paper. It is sufficient to state here that all termite protozoa are not killed in the same time at a given temperature, that is, the time-temperature relation is not constant. It is not constant even for all the protozoa of certain termites; in *Kalotermes tabogae* at approximately 35.5°C. the trichonymphids are all killed in two days (many are killed much sooner), while it is five or six days before the calonymphids are affected at all and eight to ten days before they are all killed. At 36.5°C. the trichonymphids of this termite are killed in less than twenty-four hours, while the calonymphids are not killed in three days. But *Calcaritermes*, for instance, loses its protozoa within less than twenty-four hours when incubated at 35.5°C.

After *Reticulitermes* had been freed of its intestinal protozoa (Cleveland, 1924), it was carefully studied. As a rule it died within three weeks or thereabouts after its protozoa were taken from it. It was discovered, however, that the actual length of time required for death to take place depended on the food eaten; the more decayed the food (wood) the longer it lived. When given humus or fungus-

digested cellulose (paper), it lived very much longer and would perhaps have lived indefinitely had it been properly cared for; molds, as mentioned before, kill termites when the moisture content is high and they cannot live without a certain amount of moisture. It is difficult to maintain the right amount. The best way devised so far is to keep the termites in a closed jar or flask which is connected with a moist chamber of some sort—another flask will do—by means of a small glass tube. Once the proper amount of moisture is ascertained, it may be kept indefinitely without any attention whatever. Incidentally, this same method is very useful in keeping untreated termites under close observation in the laboratory. The moisture required by different termites differs greatly; some species of *Cryptotermes* require almost no moisture at all and can live in furniture for a long time.

The fact that the incubated termites lived very much longer, if not indefinitely, on a predigested diet of humus or fungus-digested cellulose than on their normal diet of partially decayed wood indicated that the incubation in itself was not responsible for their death. Obviously, the best way to determine whether the incubation killed the termites directly or indirectly was to incubate a large number of individuals, thus freeing them of protozoa, then replace the protozoa in one portion but not in the other, then feed each portion on the same normal diet of wood under identical conditions. This was done, and all those termites in which the protozoa were restored were able to live indefinitely, while the others all died in approximately three weeks. It was thus shown that the death of the incubated termites was due to the removal or killing off of the protozoa rather than to the incubation in itself. Here, then, is an ideal case of symbiosis; neither partner (nor symbiote) can live without the other. A termite chews and swallows wood which the countless millions of protozoa living in it eat and digest for themselves and for the termite that maintains them in a gastronomical paradise—its gut.

Starvation

When termites are starved, practically all their large protozoa and many of their small ones die long before they do. The large protozoa in practically every species of termite die more quickly than the small ones. Perhaps the best way to carry out a starvation experiment is to place the termites in clean, flat-bottomed, dry glass vessels, and keep these in moist chambers. Here again the amount of moisture present is important. If there is too much or too little, the termites will not die of starvation.

More extensive observations and experiments have been carried out on the starvation of the large Pacific Coast termite, *Termopsis*, than on any other (Cleveland, 1925b). This termite contains protozoa of four genera (figs. 1–4) in fairly definite proportions and when starved, its largest protozoon, *Trichonympha campanula* (fig. 1), dies first. After about six days' starvation this protozoon disappears entirely. If the starvation is continued, the next largest protozoon, *Leidyopsis sphaerica* (fig. 2), disappears by the end of eight days. After twelve to fifteen days' starvation, the other protozoa, *Trichomonas termopsidis* (fig. 3) and *Streblomastix strix* (fig. 4), are greatly reduced in number, but it is impossible to remove everyone of them before the temites die or are greatly injured. Very few of them live more than twenty-five days.

If, after six days' starvation, which removes *Trichonympha*, the termites are returned to their normal wood diet, they are able to live indefinitely as shown in

table 1. *Leidyopsis*, then, which in untreated termites is seldom if ever present in such great numbers as *Trichonympha*, multiplies rapidly and takes the place of *Trichonympha* in number and, as we shall see later, as the principal symbiote. When both *Trichonympha* and *Leidyopsis* are removed by eight days' starvation, these termites, when returned to their normal diet, are able to live sixty to seventy days; whereas when all protozoa are removed (see table 1), termites live only three to four weeks. Evidently, then, the remaining protozoa, *Trichomonas* and *Streblomastix*, are of some value to their hosts but are not able to keep them alive indefinitely. The removal of *Trichonympha* and *Leidyopsis* destroys the symbiotic association, although not completely, and results, finally, in the death of *Termopsis* and its protozoa. This termite has certainly formed a most intimate partnership with two of its protozoa.

TABLE 1.

Results of various methods which have been employed in removing one or more genera of protozoa from the large Pacific Coast termite, Termopsis nevadensis Hagen

Every host in nature always harbors each genus. − = absent, *i.e.*, treatment killed all protozoa of this genus and + = present, *i.e.*, treatment had no effect.

METHODS OF TREATMENT	THE PROTOZOA				RESULT OF TREATMENT ON HOST WHEN FED ITS NORMAL WOOD DIET
	Trichonympha	*Leidyopsis*	*Trichomonas*	*Streblomastix*	
1. Starvation for 6 days	−	+	+	+	Lives indefinitely
2. Starvation for 8 days	−	−	+	+	Lives about 10 weeks
3. Oxygenation for 24 hours at 1 atm	+	+	−	+	Lives indefinitely
4. Oxygenation for 24 hours at 1 atm. Starvation for 6 days	−	+	−	+	Lives indefinitely
5. Oxygenation for 24 hours at 1 atm. Starvation for 8 days	−	−	−	+	Lives 3–4 weeks
6. Oxygenation for 7 hours at 1.5 atms	+	+	−	−	Lives indefinitely
7. Oxygenation for 7 hours at 1.5 atms. Starvation for 6 days	−	+	−	−	Lives indefinitely
8–13. Oxygenation, 1 atm., 72 hrs.; 1.5 atm., 9 hrs.; 2.5 atm., 2 hrs.; 3 atm., 1 hr. and 5 min.; 3.5 atm., 40 min	−	−	−	−	Lives 3–4 weeks.

Oxygenation

When termites are placed in oxygen under pressure, they lose their protozoa very quickly, indeed (Cleveland, 1925b, 1925c), and without being injured in the least. This is a better method of freeing them of protozoa than incubation or starvation. But here, as in incubation and starvation, all termite protozoa are not affected in the same way when oxygenated. Some of them lose their protozoa when confined in an atmosphere of practically pure oxygen, that is when they are changed from an atmosphere of air with approximately 20 per cent oxygen to an atmosphere of 95 to 98 per cent oxygen; while others do not lose their protozoa until they are placed in slightly more than one atmosphere of oxygen (table 2). As the oxygen pressure increases, the time required to kill the protozoa decreases rapidly until a pressure of three and a half to four atmospheres is

reached. At a pressure of fifteen atmospheres, for instance, the protozoa are killed in about half the time that they are killed when oxygenated at a pressure of three and a half atmospheres; whereas three and a half atmospheres kills them in half the time that three atmospheres does. In other words, one-half of an atmosphere between three and three and a half atmospheres cuts down the time required to kill the protozoa as much as ten or eleven atmospheres do after three and a half to four atmospheres have been reached. Obviously, then, the best oxygenation pressure to use in breaking the

TABLE 2.

Time required at various pressures of oxygen to kill all intestinal protozoa of certain termites

PRESSURE IN ATMOSPHERES	RHINOTERMITIDAE				KALOTERMITIDAE			
	Leucotermes		*Reticulitermes*		*Termopsis*		*Cryptotermes*	
	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes
1.0	24		*		72		*	
1.5	4	30	9		9		7	30
2.0	1	35	4		5		4	30
2.5	1	15	1	40	2		1	55
3.0		50		50	1	5	1	
3.5		30		30		40		35

* Not killed in ten days.

symbiosis between termites and their protozoa is somewhere between three and four atmospheres.

Work is now in progress on the relation of the protozoa of many species of termites from Panama and Costa Rica to their hosts. The work is not complete, but it is evident that some of these termites are able to live longer than three to four weeks after their protozoa have been taken from them. Many of them, however, are not. We cannot be sure that protozoa-harboring termites will die when their protozoa are killed, until actual experiment has shown that they do. Some of them, although we are inclined to doubt it, may be able to live—poorly of course—without protozoa.

Oxygenation and starvation

After having noted that oxygenation removes some termite protozoa and not others, that is, brings about a partial rather than a total defaunation, and that starvation does the same thing, it was evident that by a combination of the two partial defaunation methods interesting results might be obtained as to just what protozoa were and were not symbiotes.

The large Pacific Coast termite, *Termopsis*, as we have already seen, contains four genera of protozoa, and when oxygenated at one atmosphere, it loses *Trichomonas* (fig. 3) within twenty-four hours, but does not lose its other protozoa until three days (Cleveland, 1925b). By oxygenating this termite at a pressure of 1.5 atmospheres for seven hours it was possible to remove both *Trichomonas* and *Streblomastix* (fig. 4) from many, though never all, hosts without seriously injurying *Trichonympha* (fig. 1) and *Leidyopsis* (fig. 2). We have already noted that if this termite is starved for six days it loses *Trichonympha*, and if starved eight days it loses *Trichonympha* and *Leidyopsis*. The various protozoal combinations which may be obtained by starving and oxygenating *Termopsis* are given in table 1. Termites of each of the seven groups, and of the group with no protozoa, were fed their normal diet of wood and kept under identical conditions. Table 1 shows what happened to each group.

Either *Trichonympha* or *Leidyopsis* is able to keep its host alive indefinitely. *Trichomonas* is able to keep its host alive for a while, but not indefinitely. *Streblomastix* is of no value to its host and is not a symbiote. It may be supported either by the termites or by its protozoan neighbors, or by both. Similar work on the protozoa of other termites will perhaps show that not all termite protozoa are symbiotes.

LITERATURE LIST

BUCHNER, P. 1921. Tier und Pflanze in intrazellularer Symbiose. 462 p. Berlin: Gebrüder Borntraeger.

CLEVELAND, L. R. 1923. Correlation between the food and morphology of termites and the presence of intestinal protozoa. Amer. Journ. Hyg., 3: 444–461.

CLEVELAND, L. R. 1924. The physiological and symbiotic relationships between the intestinal protozoa of termites and their host, with special reference to *Reticulitermes flavipes* Kollar. Biol. Bull., 46: 177–225.

CLEVELAND, L. R. 1925a. The ability of termites to live perhaps indefinitely on a diet of pure cellulose. Biol. Bull., 48: 289–293.

CLEVELAND, L. R. 1925b. The effects of oxygenation and starvation on the symbiosis between the termite, *Termopsis*, and its intestinal flagellates. Biol. Bull., 48: 309–327.

CLEVELAND, L. R. 1925c. The toxicity of oxygen for protozoa in vivo and vitro: animals defaunated without injury. Biol. Bull., 48: 455–468.

DE BARY, ANT. 1879. Die Erscheinung der Symbiose.

DOFLEIN, F. 1906. Die Pilzkulturen der Termiten. Verhandl. d. Deutsch. Zool. Ges., 15: 140–149.

FULLER, C. 1910. Annals Natal Museum, 4: 235–295.

FULLER, C. 1911. The fungus food of certain termites. S. Afr. Journ. Nat. Hist., 3: 139–144.

MEYER, K. F. 1925. The "bacterial symbiosis" in the concretion deposits of certain opercutate land mollusks of the families cyclostomatidae and annulariidae. Journ. Infect. Diseases, 36: 1–107.

NUTTALL, G. H. F. 1923. Symbiosis in animals and plants. Address British Association Liverpool in 1923.

PETCH, T. 1906. The fungi of certain termite nests. Ann. Roy. Botan. Garden Peradenyia, 3: 185–270.

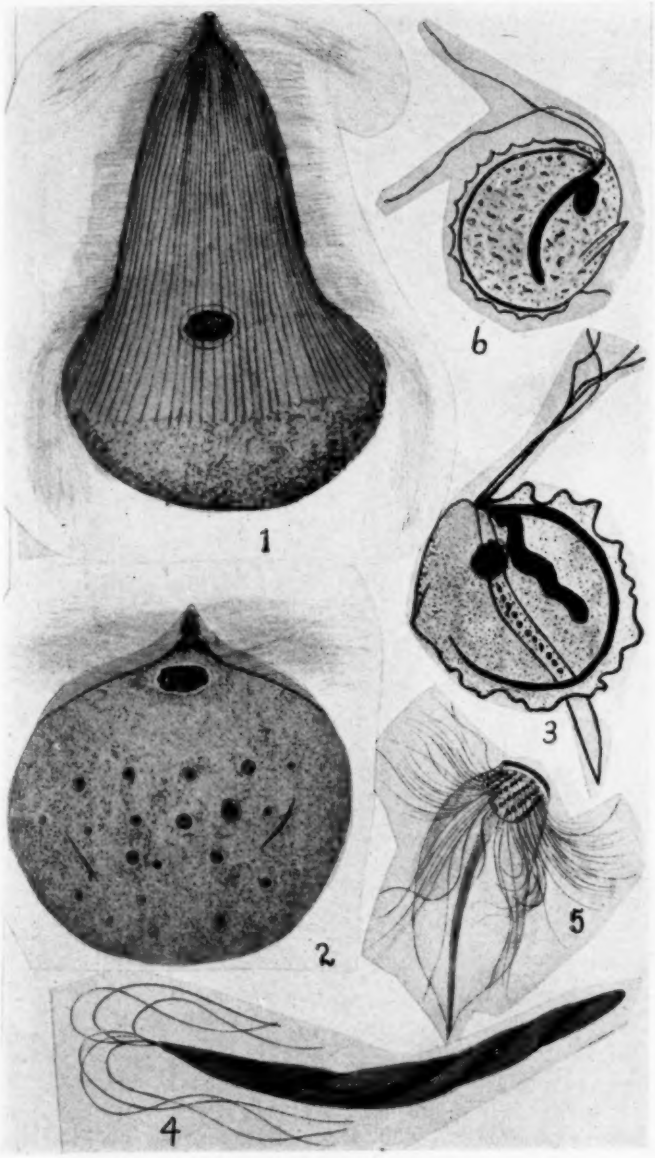
PORTIER, P. 1918. Les Symbiotes. 315 p. Paris: Masson et Cie.

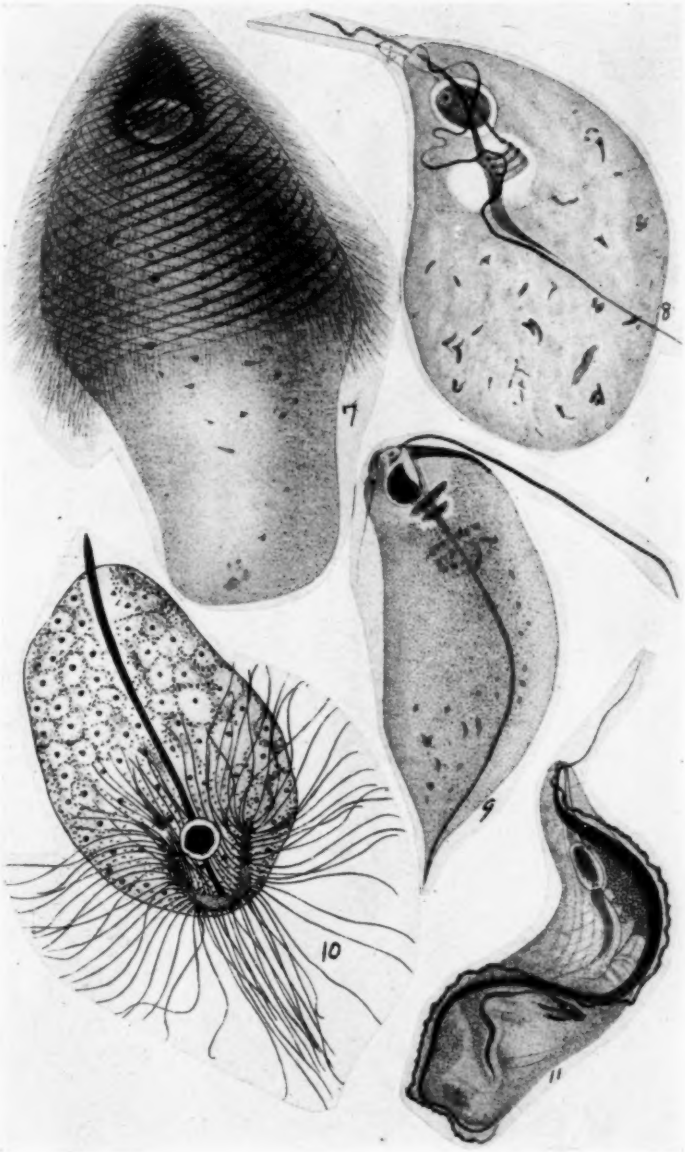
UICHANCO, L. B. 1924. Studies on the embryogeny and postnatal development of the aphidae with special reference to this history of the "symbiotic organ" or "mycetom." Philippine Journ. of Sci., 24, 143–247.

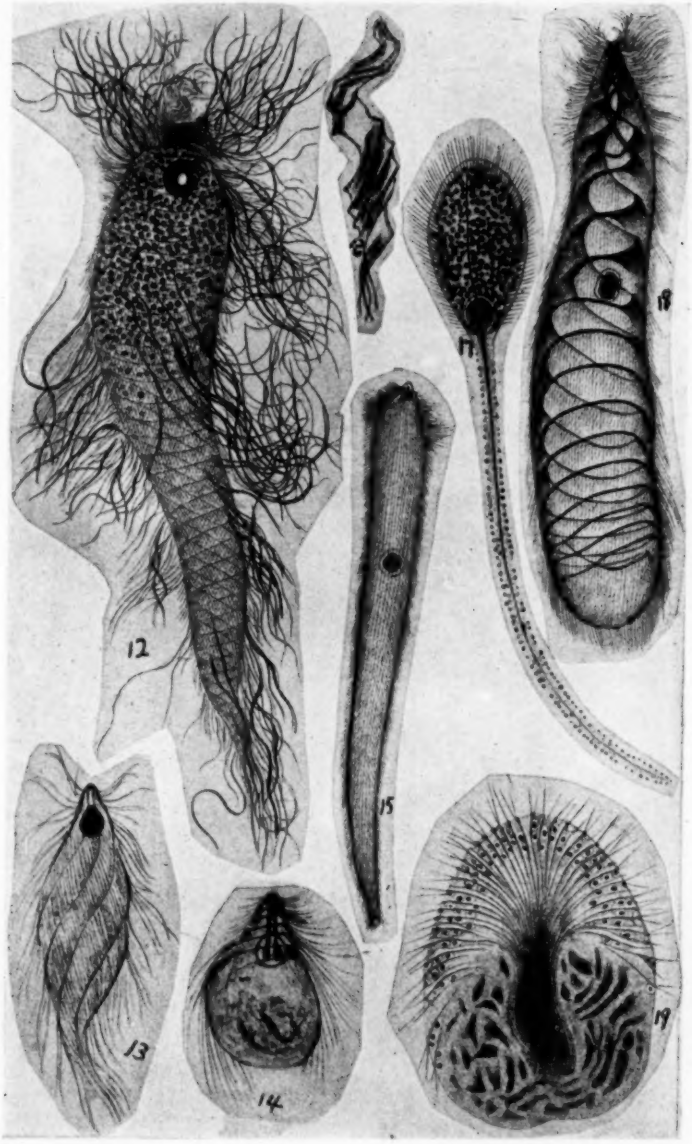
DESCRIPTION OF PLATES 1–4

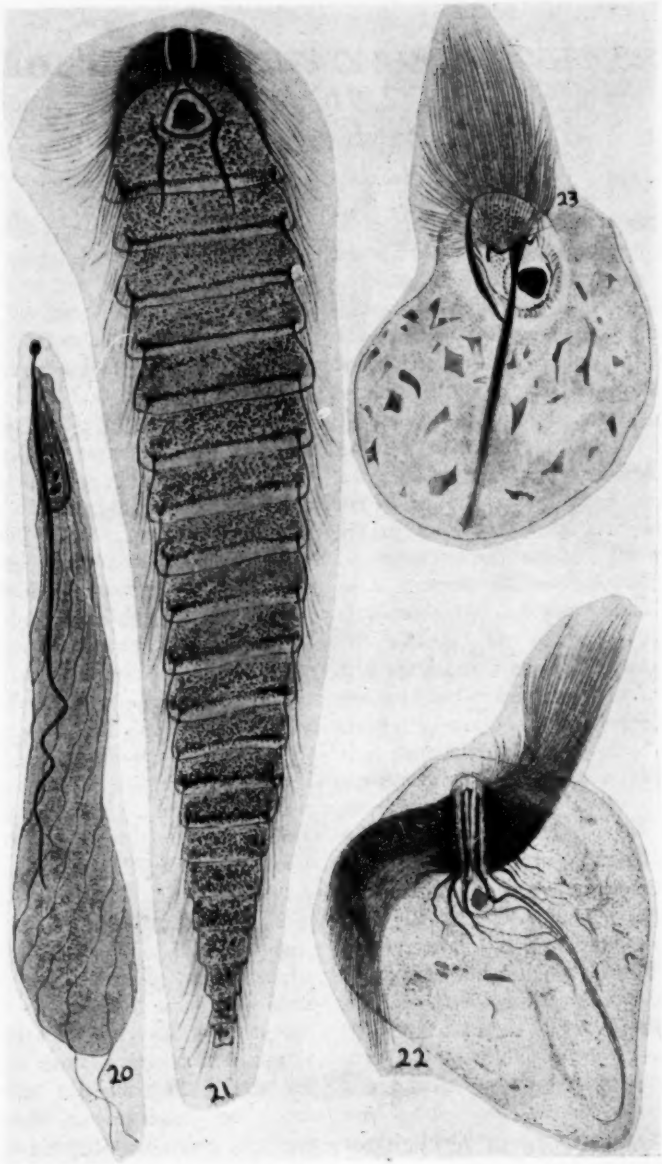
FIGURES	PROTOZOON	TERMITE FOUND IN	LOCALITY
1	*Trichonympha campanula*	*Termopsis angusticollis*	Pacific Coast, U. S. A.
2	*Leidyopsis sphaerica*	*Termopsis angusticollis*	Pacific Coast, U. S. A.
3	*Trichomonas termopsidis*	*Termopsis angusticollis*	Pacific Coast, U. S. A.
4	*Streblomastix strix*	*Termopsis angusticollis*	Pacific Coast, U. S. A.
5	*Microjoenia hexamitoides*	*Reticulitermes lucifugus*	Italy
6	*Districhomonas termitis*	*Archotermopsis wroughtoni*	India
7	*Holomastigotoides hemigymnum*	*Coptotermes lacteus*	Australia
8	*Macrotrichomonas pulchra*	*Glyptotermes parvulus*	Chile
9	*Devescovina glabra*	*Cryptotermes havilandi*	Chile
10	*Joenopsis cephalotricha*	*Archotermopsis wroughtoni*	India
11	*Pseudotrypanosoma giganteum*	*Porotermes adamsoni*	Australia
12	*Spirotrichonymphella pudibunda*	*Porotermes adamsoni*	Australia
13	*Holomastigotes elongatum*	*Reticulitermes lucifugus*	Italy
14	*Microspironympha porteri*	*Reticulitermes flaviceps*	Japan
15	*Pseudotrichonympha hertwigi*	*Coptotermes sjöstedti*	French Guinea
16	*Dinenympha rugosa*	*Reticulitermes speratus*	Formosa
17	*Stephanonympha sylvestri*	*Kalotermes grassi*	Chile
18	*Spirotrichonympha elongata*	*Schedorhinotermes intermedius*	Australia
19	*Diplonympha foai*	*Glyptotermes parvulus*	Gold Coast
20	*Pyrsonympha grandis*	*Reticulitermes speratus*	Formosa
21	*Teratonympha mirabilis*	*Reticulitermes flaviceps*	Formosa
22	*Saturojoenina mirabilis*	*Epicalotermes aethiopicus*	Eritrea
23	*Joenina pulchella*	*Porotermes adamsoni*	Australia

Figures 1, 2, 4 after Kofoid and Swezy; 5, 7, 8, 9, 10, 11, 12, 15, 17, 18, 19, 22, 23 after Grassi; 6, 10 after Cutler; 13, 14, 16, 20, 21 after Koidzumi.


1
2
3
4
5
6


7
8
9
10
11


12
13
14
15
16
17
18
19


23
20
21
22

EXPERIMENTAL STUDIES ON MORPHOGENESIS IN THE NERVOUS SYSTEM

By S. R. DETWILER

Zoölogical Laboratory, Harvard University

THE application of the experimental method to the study of the developing nervous system has yielded results which have extended greatly our knowledge regarding certain aspects of differentiation. One of the outstanding achievements made by the use of this method was the solution of the well known question of the genesis of the nerve fiber. The neurone theory of His ('86, '87) and Forel ('87), which seemed incapable of being satisfactorily demonstrated by the method of direct observation, was given firm and conclusive support mainly through the experimental researches of Harrison ('04a, '06, '07a, '07b, '10a, '10b).

The question of the origin of the nerve fiber was first tested experimentally by Harrison ('04a, '06) and by Braus ('05). Harrison removed the ganglionic crest cells from frog embryos, and found that the embryos developed without sensory nerves and ganglia, but that motor nerves were present. These were devoid of sheath cells. He also showed that, when the ventral half of the embryonic spinal cord was removed, but with the dorsal part of the cord and ganglionic crest cells intact, the larvae were devoid of the motor nerves. His results clearly indicated that the nerve fiber does not have its origin in the sheath cells, but that it grows out from a single ganglion cell, with which it remains in continuity throughout life. A full account of researches on the histogenesis of the nerve fiber has recently been published by Harrison. (Jour. Comp. Neur., vol. 37, 1924.)

Braus made the interesting discovery that the amphibian limb rudiment has the ability to undergo differentiation independent of its normal organic environment ('04), and that when transplanted to an abnormal situtation it may acquire nervous connection with the central nervous system of the host and even exhibit spontaneous movements. He took advantage of limb grafting as an experimental method for the study of a number of fundamental questions concerning the development of the nervous system. He did not believe that the nerves, which developed within a transplanted extremity, grew in from the central nervous system of the host, but that the peripheral portions of the nerves developed *in situ* within the appendage and secondarily made connections with the nervous system of the host. Although all of Braus' experiments were ingenious in type, he endeavored to support Hensen's ('64) protoplasmic bridge theory, according to which protoplasmic bridges are supposed to be left everywhere between dividing cells of the embryo, so that when nerves begin to differentiate there is a complex system of protoplasmic connections within the organism. Those which function as conduction pathways are supposed to differentiate into nerve fibers, whereas the remainder ultimately disappear.

Banchi ('06) also carried out limb

transplantation experiments in connection with the question of nerve origin and likewise interpreted his results in support of the Hensen theory. In the limb grafting experiments of Harrison ('07a) and Gamelli ('06), evidence was brought to bear against the Hensen theory, and the outgrowth theory was given valuable experimental support. Whereas Braus claimed that aneurogenic limb buds (those taken from nerveless larvae) did not acquire nervous connection with the host—since they were disconnected from the nerve centers during the critical period, Harrison's experiments showed that aneurogenic as well as eneurogenic limb buds (those taken from normal larvae) became supplied with peripheral nerves. Harrison made a much more crucial experiment in grafting a limb rudiment from a normal embryo to one which had previously been deprived of its central nervous system. In such cases the developing appendage was devoid of nerves. This indicated, therefore, that nerves which develop within a grafted appendage, grow into it from the central nervous system of the host.

Whereas the results of Harrison's limb grafting experiments left little doubt in the minds of most neurohistologists as to the validity of the outgrowth theory, nevertheless, the advocates of the Hensen school still demanded more rigorous proof. This was soon after furnished by the tissue culture experiments of Harrison ('10a) in which he explanted neuroblasts of frog embryos into clotted lymph and observed with the eye the developing nerve as a protoplasmic outgrowth from a single ganglion cell, a concept which had already gained considerable footing through the careful investigations of His ('86, '87, '88, '90), Ramón y Cajal ('90, '92, '94, '06) and v. Lenhossék ('92, '95, '06). Harrison's results were corroborated by Burrows ('11) and Lewis ('11) and the 'neurone' as the genetic and morphological unit of the vertebrate nervous system became firmly fixed. With the establishment of this concept great attainments have been made in the fields of nerve regeneration and transplantation, and our knowledge of reflex pathways has grown rapidly.

THE INFLUENCE OF THE PERIPHERAL FIELD ON NEURONE DEVELOPMENT

Although it seemed clear from the experiments on explantation of primitive ganglion cells, that the initial outgrowth of the nerve fiber can proceed from the neuroblast independently of any functional requirements on the part of the endorgan, no especial attempt was made in these experiments to study the effects of normal organic stimuli upon the extent of neuronic differentiation. The results of experiments directed towards the solution of this question (Braus, '06; Burr, '16a; Dürken, '11; Shorey, '09, '11) indicated that in the absence of certain peripheral areas, the nerve centers normally supplying those areas undergo hypoplastic development, supposedly from the lack of the peripheral functional demands which normally activate their complete development. Braus (op. cit.) found as a result of excision of the forelimbs of *Bombinator* prior to the outgrowth of the brachial plexus, that the plexus in larvae preserved ten days after the operation was as well developed as the normal, and that no reduction in the size of the ventral horn areas could be detected. Observations, however, on operated larvae, which were kept alive until just before metamorphosis, showed that the brachial plexus was reduced in size, as were also the ventral horn areas ordinarily supplying the limb. Braus

interpreted his results in accordance with the general developmental theory of Roux ('85) in stating that the development of the central nervous system can be divided into two periods: the first, in which growth and differentiation are independent of functional activity; and the second, in which further differentiation and growth continue only under the influence of this factor.

Miss Shorey ('09) carried out extirpation experiments on the limb rudiments of *Amblystoma* and the chick, and claims

plied in the culture medium. As a result of her observations she concluded that motor nerve growth is not only entirely dependent upon the presence of muscles, but that no neuroblasts can differentiate unless under the stimulus supplied by the functional endorgan or the metabolic products of the same. On the basis of her observations she opposed Harrison's conclusions that the outgrowth of the nerve fiber can proceed independently of any functional requirements on the part of the endorgan.

TABLE 1

Showing the effects of removal of the limb and the activity of the transplanted limb upon the development of peripheral afferent neurones

GANGLION CONNECTED WITH NORMAL INTACT LEFT LIMB			GANGLION WITH RIGHT LIMB REMOVED			GANGLION CONNECTED WITH RIGHT LIMB TRANSPLANTED TO A HETEROTOPIC POSITION			GANGLION NOT CONNECTED WITH EITHER LIMB			RATIOS	
Number of spinal ganglion	Number of cells counted	Weight of the model in grams	Number of spinal ganglion	Number of cells counted	Weight of the model in grams	Number of spinal ganglion	Number of cells counted	Weight of the model in grams	Number of spinal ganglion	Number of cells counted	Weight of the model in grams	Cell number	Weight
3	1,725	30.4841	3	850	12.9748							0.492	0.425
4	1,430	26.1604	4	685	9.7311							0.479	0.372
5	955	16.8798				5	1,195	22.2939				1.25	1.32
						6	1,171	26.5422	6	693	12.6600	1.69	2.09
						7	1,084	21.7634	7	710	11.7366	1.51	1.85
									9*	754	15.5456		
									9†	773	17.2328	1.02	1.10

* Left ganglion.
† Right ganglion.

to have found marked deficiencies in the peripheral nerves as well as in the ventral horn areas subsequent to the removal of the appendicular musculature. Also, the degree of the defect was said to be more or less proportional to the extent of the peripheral destruction. In a later work ('11) in which she explanted neuroblasts in a variety of culture media, she claimed that axone outgrowth ensued only in those cases in which beef extract (metabolic products of muscle) was sup-

HYPERPLASIA IN SENSORY GANGLIA

It was in connection with the above question that the original limb experiments of the writer (Detwiler, '20a, '20b) were carried out. Rather than to test the effects of the destruction of peripheral areas on the developing neurones, experiments were devised to test whether or not, by overloading the periphery at a given region, the corresponding peripheral neurones can be induced to undergo hyper-

normal (hyperplastic) development to meet the added functional requirements imposed upon them. Accordingly, the right anterior limb rudiment of *Amblystoma* embryos was transplanted in the same embryo and at distances varying from one to seven somites caudal to the normal situation (autoplastic grafts). In this way it became possible to study the effects of the development and function of limbs so placed upon the neurones supplying them. Observations on the functional responses of the transplanted limbs as well as the segmental nerve supply have been published previously in tabular form (Detwiler, '20b, tables 1 and 2). It may not be redundant here to state that limbs which were transplanted from one to three segments caudal to the normal position were found to receive nearly their entire nerve supply from the original limb levels of the cord (third, fourth, and fifth segments). Limbs which were transplanted a distance of more than three body segments caudal to the normal position received the bulk of their nerve supply from segments of the cord just posterior to the original limb levels (sixth, seventh and eighth segments). The nerves of these levels normally supply afferent fibers to the body integument and efferent fibers to the muscles of the body wall. Throughout these experiments there was evident a marked tendency for the transplanted limbs to receive innervation from the normal limb levels of the cord.

The most instructive cases of the limb experiments were those in which the limbs were shifted caudally the distance of four body segments (fig. 15). Limbs so placed were typically found to be innervated from a plexus composed of the fifth, sixth, and seventh segmental nerves, the fifth of which is a normal limb nerve. Limbs so innervated exhibited a high degree of activity which was coordinated with the contralateral extremity. Microscopical examination of such cases showed that the strange segmental nerves (sixth and seventh) contributing to the grafted limb were larger than the contralateral nerves which had, of course, no connection with

TABLE 2

Showing number of cells counted in twenty consecutive transverse sections of the right half of the spinal cord at the level of the third, fourth, and fifth segmental nerves respectively (anterior limb levels)

CONDITIONS	CASES	III NERVE LEVEL	IV NERVE LEVEL	V NERVE LEVEL	CELLULAR RATIO BETWEEN III AND V NERVE LEVELS
Normal	AS_4	3464	2796	2346	III:V 1.48:1.00
Limb excised, spinal cord intact	AS_{42}	3904	2803	Connected with limb 2352	1.66:1.00
Seventh, eighth, and ninth spinal segments substituted for the third, fourth, and fifth, respectively	$TrSC_{127}$*	3130	2600	2500	1.25:1.00
	$TrSC_{129}$*	3043	2973	2728	1.11:1.00
Limb level of spinal cord (third, fourth, and fifth segments) reversed	$ReSC_1$†	3500	2593	2213	1.62:1.00
	$ReSC_{10}$†	3265	2809	2326	1.40:1.00

* In cases $TrSC_{127}$ and $TrSC_{129}$, the third, fourth, and fifth nerve levels represent, respectively, the transplanted seventh, eighth, and ninth.

† In cases $ReSC_1$ and $ReSC_{10}$, the third nerve level represents the reversed fifth, whereas the fifth nerve level represents the reversed third.

a limb. This enlargement was found to be due to a hyperplasia of the sensory neurones. Evidence of sensory hyperplasia was not only suggested by the obvious differences in the size of the spinal ganglia (figs. 13, 14) and the posterior roots, but was also quantitatively estimated by making a numerical count of the sensory ganglion cells (table 1).

Excision of the limb rudiment was also found to result in a 52 per cent hypoplastic development of the sensory neurones of the limbless nerves (table 1). From the fact that the percentage of weight reduction in the posterior roots exceeded that of the ganglia, the results also indicated that in addition to a hypoplasia, a slight atrophy of the afferent neurones ensued. No exact quantitative observations have been made on the size changes in the sensory neurones disconnected with the normal limb or in those connected with a transplanted limb.

As striking as the sensory responses have been to decreases and increases in the peripheral field, no experiments have as yet been completed to ascertain at what period in the development of the embryo these reactions set in or terminate, though investigations concerning these questions are under way. Furthermore, the question as to whether the sensory hyperplasia in nerves connected with a transplanted limb is entirely related to the integumentary increase is one which also needs investigation. An analysis of this situation meets with difficulty since when spinal nerves are brought into relation with a transplanted limb, they not only have a greater integumentary area to supply, but by reason of the added musculature (limb and shoulder muscles) it is reasonable to expect that there should be an augmentation in the number of proprioceptive fibers to the muscles. Since it has been impossible to distinguish between proprioceptive and exteroceptive neurones, it is difficult to say how much of the hyperplasia in the ganglia can be ascribed to integumentary increase. It may be possible, however, to analyze the situation further as a result of experiments which are under way. These have been devised in a way to diminish the integumentary area on the embryo without affecting a diminution in the musculature. By so doing it may be possible to obtain a rough estimate of the part played by exteroceptive and by proprioceptive neurones in hyperplased ganglia connected with a limb.

Whereas the limb transplantation experiments have shown a marked sensory reaction (hyperplasia) to the peripheral overloading, no evidence as yet of a similar response on the part of the efferent neurones has been obtained either from a comparative study of the motor roots or from a numerical comparison of the motor nerve cells in both halves of the spinal cord at the levels involved. Neither as a result of the excision of the limb was there any measurable evidence of a hypoplastic development of the efferent fibers in the limb nerves ('20a, '23a), though such nerves actually did suffer a reduction in size.

A study of size changes in the primary brachial motor neurones following limb excision in *Amblystoma* embryos has recently been made (Detwiler and Lewis, '25) with the interesting observation that bilateral excision of the anterior limb rudiments causes a greater reduction in the size of the primary brachial motor neurones than when only one limb is excised. In the latter case the average area of the median plane of section of the motor horn nuclei (as estimated by the polar planimeter) shows a reduction of 8 per cent. The volume reduction of the motor roots is 24 per cent. Following bilateral ex-

cision of the limb rudiments the average area of the median plane of section of the motor horn nuclei shows a reduction of 20 per cent and the volume reduction in the motor roots is 32 per cent.

These results suggest that the size of the efferent neurones, in addition to being affected by the completeness of their functional connection with the peripheral field, is also dependent upon reflex connection with local commissural neurones of the opposite side of the cord. The exact way in which commissural neurones influence growth in the motor horn cells on the opposite side of the cord we are not yet in a position to state. The results indicate, however, that growth and function in groups of neurones may affect growth processes in others regardless of whether or not the latter be fully or only partly in connection with the peripheral field.

From the observed facts that proliferation in the primary brachial motor neurones is apparently unaffected by the excision of the limb rudiment, and that efferent neurones from atypical regions of the spinal cord fail to undergo hyperplastic reactions when in connection with a terminally increased musculature (transplanted limb), it is obvious that the extent to which brachial efferent neurones shall develop is not primarily under the control of the peripheral musculature. This conclusion is not in correspondence with the results of Miss Shorey's experiments, nor with the reflex circle idea of Bok ('17). Vermuelen (communicated by Bok) also reports a human monster in which a duplication of the tongue musculature was accompanied by a duplication of the hypoglossal nuclei in the brain stem. It is difficult to harmonize the observations of Vermuelen and Bok with my own results. It would seem that if increased functional activity on the part of the peripheral musculature had any effect upon proliferation of motor neuroblasts, manifestations of a definite sort should be forthcoming in the limb transplantation experiments, yet in no case so far studied, whether the transplanted limb was large or small, functional or useless, single or double, has there ever been found measurable evidence of motor hyperplasia such as has been almost without exception on the part of the sensory nerves.

The limb grafting experiments have indicated clearly then that the principal agencies involved in neuroblastic proliferation of sensory and of motor nerves are not the same, and that factors other than the functional activity of the end organ must combine in determining the extent of motor cellular differentiation within the spinal cord.

PROLIFERATION OF CELLS IN THE CENTRAL NERVOUS SYSTEM

It was suggested as a result of the limb experiments that if the extra-limb segments of the cord, which are capable of producing only limited movements in transplanted limbs, could be substituted for the limb region of the cord, it would present a condition whereby it would be possible to study more favorably the ability of such extra-limb segments to execute normal movements when in connection with the proper central reflex pathways. Also the substituted portion of the cord would be subjected to all the stimuli which normally produce the typical proliferation of nerve cells in this region.

Experiments were then carried out in which the anterior limb region of the spinal cord (third, fourth, and fifth segments) were excised and replaced by a more caudal unit comprising the seventh, eighth, and ninth segments from another embryo ('23a). A typical example of

such a case with a composite spinal cord is shown in figure 18. A complete report of these experiments has been published elsewhere (Detwiler, '23a) and reference to them will be made here only in so far as they bear upon the questions under consideration. Aside from the fact that in 50 per cent of these cases with composite spinal cords, normal limb activities ensued, it was found that the grafted segments of the cord in their new situation (anterior limb region) underwent an increased cellular proliferation approximately equal in extent to that which characterizes the normal anterior limb levels. The results of a cellular count from the spinal cord of a normal larva of approximately fifty days of age and from an experimental animal are shown in figures 1 and 2 respectively. From figure 1 it is seen that throughout the spinal cord from the level of the third nerve to the level of the ninth there is a gradient of cellular proliferation which diminishes down the cord. In the experimental case (fig. 2) the same is to be noted, yet here the greater cellular proliferation characterizing the anterior limb region of the cord (third, fourth, and fifth segments) as compared with the more caudal region has proceeded from a unit of cord which typically occupies the caudal position (seventh, eighth, and ninth segments).

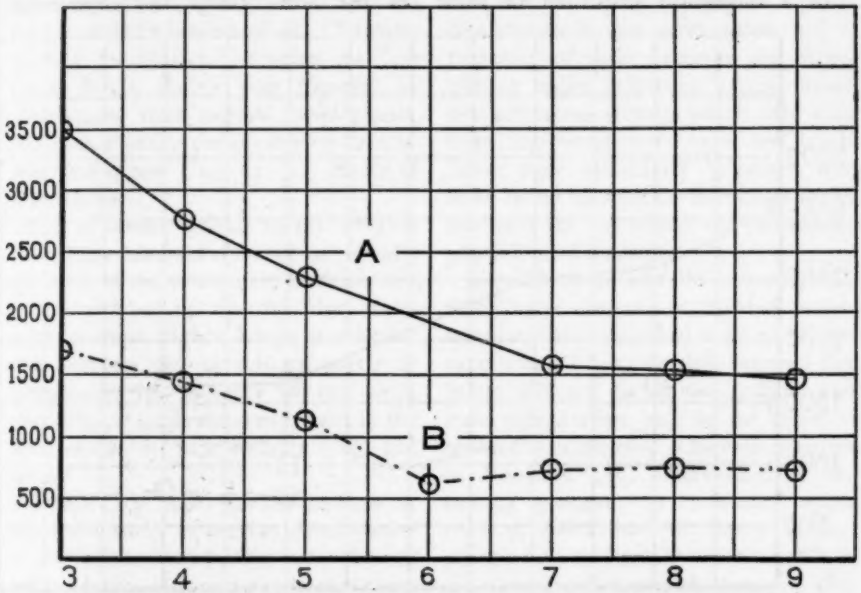


FIG. 1. GRAPH SHOWING THE EXTENT OF CELLULAR PROLIFERATION WITHIN THE RIGHT HALF OF A NORMAL SPINAL CORD FROM THE LEVEL OF THE THIRD NERVE TO THE LEVEL OF THE NINTH (CURVE A), AND THE EXTENT OF SENSORY PROLIFERATION IN THE CORRESPONDING SPINAL GANGLIA (CURVE B)

Abscissae designate the respective spinal segments (three to nine); ordinates represent in Curve A, the number of cells counted in twenty consecutive transverse sections of the spinal cord at the levels of each nerve, and in B, the number counted in each corresponding right ganglion. The third, fourth, and fifth segments are normally connected with the limb.

Since extirpation of the anterior limb was found to have no effect upon cellular proliferation in the cord ('23a), the

marked hyperplasia observed in the grafted unit of spinal cord appears to have no primary relation to the appendicular musculature. This conclusion has received further support from a series of experiments in which a cellular hyperplasia of similar magnitude occurred in the grafted unit of cord in the absence of the limb ('24).

In a theoretical discussion of these results it was suggested that the increased cellular proliferation in the transplanted seventh, eighth, and ninth segments in their grafted position may be the result of their being under the influence of a greater number of central longitudinal pathways descending from higher levels, such as the fasciculus longitudinalis medialis and the tractus bulbospinalis (Herrick, '14). This viewpoint seemed further justified as a result of experimental end-for-end reversal of the limb region of the spinal cord (third, fourth, and fifth segments) which resulted in the development of a normal pattern of the spinal cord and peripheral nerves from the inverted segments (Detwiler, '23b).

Under normal conditions more nerve cells develop in the third segment than in the fifth. Under the experimental conditions the fifth segment, which occupies the position of the third, is found to undergo hyperplastic development similar to the normal third, whereas the third segment when moved caudally to the position of the fifth, undergoes decreased proliferation so as to typify the normal fifth segment (table 2).

From the constancy in the extent of proliferation within the various seg-

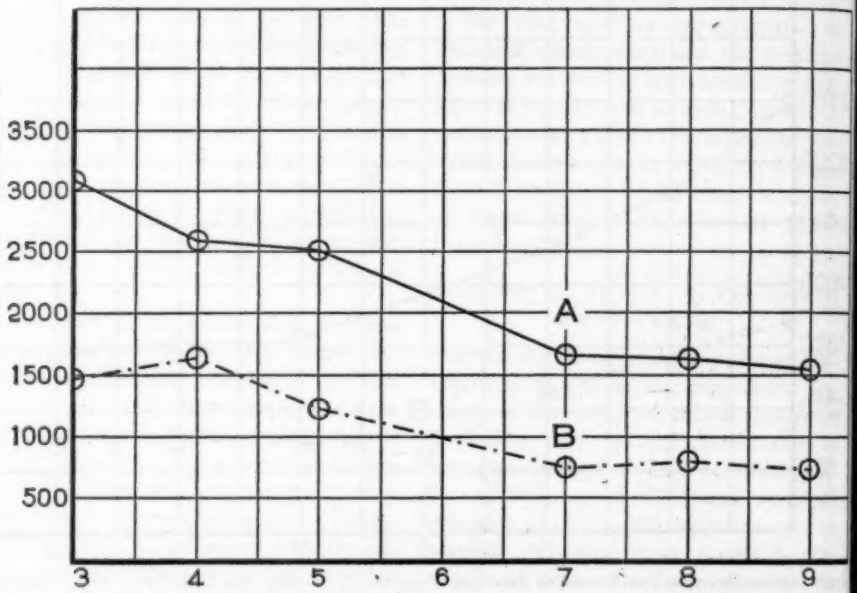


FIG. 2. GRAPH SHOWING THE EXTENT OF CELLULAR PROLIFERATION WITHIN THE RIGHT HALF OF THE SPINAL CORD (CURVE A) AND IN THE RIGHT SPINAL GANGLIA (CURVE B) IN EXPERIMENTAL CASE $TrSC_{137}$

Normal anterior limb region of the cord excised (third, fourth, and fifth segments) and replaced by the seventh, eighth and ninth segments from another embryo. Abscissae and ordinates as in figure 1.

ments of the limb region of the cord under both experimental conditions, the accumulated evidence seems to bear out the idea that the degree of proliferation which is reached in these levels of the cord is commensurate with the number of longitudinal conduction fibers normally terminating therein for the coordinate control of the appendicular reflexes. If such were the case is it possible that we have here an exemplification of the "Stimulogeneous Fibrillation" concept of Bok ('15)? Bok's theory was founded on observations from normal development, and forms much of the anatomical foundation for Kappers' ('17, '21, '22) theory of neurobiotaxis.

It is of course difficult to say whether the cellular increases observed are actually the result of the stimulative influence of a greater number of internal fiber tracts entering these higher levels or whether such increases can be referred partly or entirely to the influence of the axial physiological gradients as suggested in the work of Child ('21), Bellamy ('19) and others.

Coghill ('23, '24b) has shown from an exhaustive study of nervous development in *Amblystoma* (non-motile, early flexure, coil and early swimming stages) that there are localized regions where differentiation and proliferation of cells take place according to a very definite pattern, and that acceleration of these processes in different regions of the central nervous system is definitely related to the development of the behavior pattern. He further shows that one process tends to become accelerated where the other is retarded, and that both take place in the form of waves which probably pass cephalocaudad through the brain and spinal cord. His work is of great importance since it distinguishes in a quantitative way between the two processes of proliferation and differentiation of the nervous elements. He has clearly brought out fundamental morphological differences between the two as well as the general physiological significance of these two phases of growth. His observations afford convincing evidence that in early stages of development, the resolution of indifferent cells into neuroblasts, the early proliferation of these neuroblasts and the regional localization in the acceleration and retardation of these processes are accomplished under influences which precede any activating stimuli which may come from ingrowing nerve roots and tracts. These more elementary influences seem to be bound up with the hereditary organization of the protoplasm and the primary physiological gradients.

In referring to the cellular hyperplasias which were observed in the transplanted spinal segments described in my spinal cord experiments ('23a), Coghill suggests that injury inflicted during the grafting may have played some part in the extensive proliferation observed. He calls attention to Hooker's '(15) observations on the healing processes in transected spinal cords of *Amblystoma* and *Rana*. Hooker observed a rapid proliferation of cells in the vicinity of the wound seven days after the operation. In a later work Hooker ('23) reported that injuries to the cord produce proliferation by mitosis of the cells of the mantle layer. Coghill's suggestion, therefore, partly rests upon Hooker's observations.

A full discussion of this matter is taken up in a separate paper (Detwiler, '25c) in which it is shown that injury inflicted during the grafting in the spinal cord segments probably plays no significant rôle in the observed hyperplasias. In this connection the experiments dealing with the end-for-end reversal of the limb region of the cord are instructive. Here

the third segment has been replaced by the fifth and the latter in turn has been replaced by the third. Reference to table 2 will show that the number of cells counted in the transplanted third segment in both cases was slightly lower than in the normal fifth, and in the transplanted fifth segment the number (case ReSC 10) is approximately 200 less than in the normal third. From the fact that in three instances out of four transplanted segments, proliferation is slightly less than that characterizing the normal, injury must be looked upon as being an ineffectual factor in the results. The same applies to the experiments in which the first five segments of the cord were excised and replaced by a unit of central nervous system consisting of the medulla and the anterior two segments of the cord (Detwiler, '25a). Here the transplanted medulla underwent hypoplastic development whereas the spinal portion of the graft underwent hyperplastic development. Since both parts of the graft were subjected to the same injury it is difficult to imagine any significant effects due to this factor.

The data at hand indicate, therefore, that hyperplasias occurring in grafted units of spinal cord cannot be ascribed to the stimulating effects of injury.

Coghill ('24b) shows (his figs. 7 and 8) that in early stages of development in *Amblystoma*, cell proliferation and differentiation are associated in the growth of the spinal cord. He finds that the seventh, eighth, and ninth segments which I transplanted into the brachial region, and which underwent marked hyperplasia, fall well within the range of relatively high potentiality of both proliferation and differentiation. Since the tenth, eleventh, and twelfth segments fall beyond the limits of so great a potentiality, Coghill raises the question as to whether these latter segments would have the same capacity for growth in the brachial region as did the seventh, eighth, and ninth. Herrick ('25) also calls attention to this point and he says (page 122):

> It should be borne in mind, however, that Coghill has shown in *Amblystoma* larvae of the stages immediately subsequent to those upon which Detwiler operated, the third, fourth and fifth segments of the cord are in a field of rapid cell proliferation which seem to be an expression of cephalo-caudad pulses of growth whose underlying causes have not yet been determined, and the matter evidently needs further investigation.

He further says,

> The relative part played here by the primary physiological gradients and the influence of ingrowing fiber tracts remains to be determined.

This question is open, at least in part, to an experimental analysis which will form the subject of future investigations. By comparing proliferation in the tenth, eleventh, and twelfth segments with that in the seventh, eighth, and ninth, both under normal conditions and when transplanted into the more cranial positions, greater possibilities will be afforded to determine more definitely than hitherto the extent to which various agencies are affecting the normal growth processes.

In further consideration of the possible stimulative effects of fiber ingrowth upon cellular proliferation, attempts have been made to disconnect descending bulbar tracts from the spinal cord by interposing, in the developing embryo, mechanical obstacles such as thin sheets of celloidin between the medulla and the cord. It was the purpose to test out whether in the absence of the invasion of such tracts into the cord, proliferation therein would suffer incompletion. These experiments so far have been unsuccessful.

Recently I have published the results of a series of experiments ('25a) in which

a marked influence of the medulla on proliferation in the anterior end of the cord was obtained. Advantage was taken of Herrick's ('14) observations that in Amblystoma certain descending tracts arise in the motor tegmentum of the medulla and pass into the ventral funiculi of the same and the opposite sides of the cord (bulbo-spinal tracts). It was designed here to augment the possible stimulating effects of such descending fibers by interpolating an additional medulla just caudal to the normal.

In these experiments the first five segments of the cord were excised and replaced by a unit of central nervous system including the caudal end of the medulla and the first two segments of the cord. Under the new conditions the first three segments of the cord are replaced by the extraneous medulla and the fourth and fifth segments are replaced by the grafted first and second (figs. 3, 4).

The results of these experiments showed a more extensive proliferation of cells in the spinal segments just caudal to the transplanted medulla than occurs under normal conditions. The cellular increase was most marked in the transplanted first and second segments and became less marked in successive segments (cf. figs. 5 and 6). The cellular increases in the grafted cord segments have been looked upon as resulting from the added stimulative effects brought about by the augmentation in the descending bulbar tracts arising in the extraneous medulla, and passing ventrally into the cord.

In discussing the question of the action of gradients in the process of growth, Herrick ('25) has looked upon this cellular increase in the cord as being the direct result of the introduction of a new center of dominance into an atypical place.

The general interpretations which were made as a result of the medulla transplantations became subject to certain modifications in consequence of further experiments (Detwiler, '25d). These involved the removal of the fourth, fifth, and sixth segments of the spinal cord and replacing them by a unit of cord comprising the first three segments from another embryo (fig. 20). In several cases the third, fourth, and fifth segments were excised instead of the fourth, fifth, and sixth. Under the new conditions the grafted first and second segments of the cord occupy the positions of the fourth and fifth respectively. This position of the transplanted first two

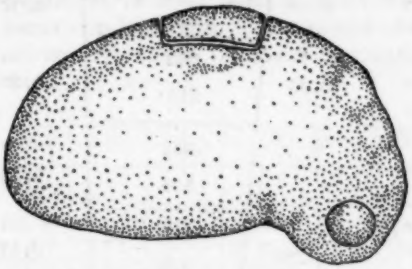


FIG. 3. OUTLINE DRAWING OF *Amblystoma* EMBRYO, SHOWING REPLACEMENT OF FIRST FIVE SEGMENTS OF THE SPINAL CORD BY A UNIT OF NEURAL TUBE COMPRISING THE MEDULLA AND THE FIRST TWO SPINAL SEGMENTS FROM ANOTHER EMBRYO. X 10

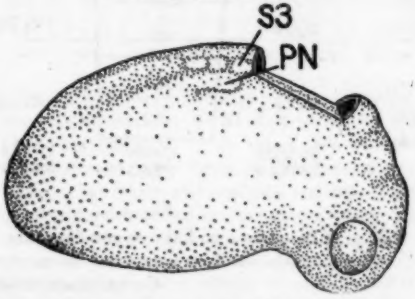


FIG. 4. OUTLINE DRAWING OF *Amblystoma* EMBRYO, SHOWING EXCISED REGION (MEDULLA AND FIRST TWO SPINAL SEGMENTS) WHICH WAS GRAFTED TO EMBRYO SHOWN IN FIGURE 3. X 10

S3, third somite; PN, pronephros

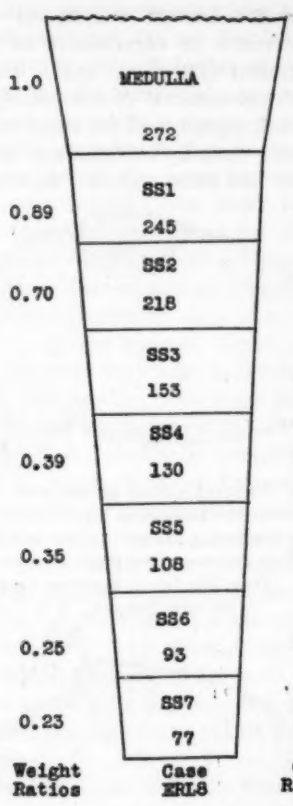


FIG. 5

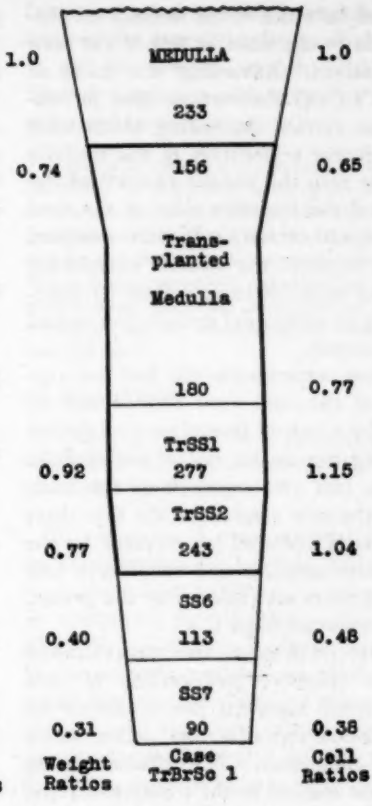


FIG. 6

FIG. 5. A DIAGRAMMATIC PLAN OF THE CELLULAR REGION OF THE CENTRAL NERVOUS SYSTEM OF A NORMAL *Amblystoma* LARVA FROM THE LEVEL OF THE ACOUSTIC (EIGHTH CRANIAL) NERVE TO THE CAUDAL LIMIT OF THE SEVENTH SPINAL SEGMENT

The central figures express the average number of cells per section as obtained by counting the number in the right halves of ten consecutive transverse sections through a known region for each segment represented. The weight ratios are based on the weights in grams of unassembled wax models of thirty consecutive transverse sections through the medulla and the various spinal segments indicated. SS_1-SS_7, first seven spinal segments.

FIG. 6. A DIAGRAMMATIC PLAN OF THE CELLULAR PORTION OF THE CENTRAL NERVOUS SYSTEM OF *Amblystoma* LARVA TrBrSC1, FROM THE LEVEL OF THE ACOUSTIC (EIGHTH CRANIAL) NERVE TO THE CAUDAL LEVEL OF THE SEVENTH SPINAL SEGMENT

The first five segments of the spinal cord were excised and replaced by a unit of neural tube comprising the greater portion of the medulla and the first two spinal segments ($TrSS_1$ and $TrSS_2$). The added medulla occupies the region of the normal first, second and third spinal segments. The transplanted first and second segments occupy the position of the fourth and fifth, respectively (v. fig. 3). The central figures express the average number of cells per section as obtained from counting the number in the right halves of ten consecutive transverse sections through the same regions as used in the study of the normal individual (cf. fig. 5). Weight ratios obtained in the same manner as outlined in explanation of figure 5. $TrSS_1$ and $TrSS_2$, transplanted first and second spinal segments; SS_6 and SS_7, normal sixth and seventh segments.

spinal segments corresponds to that occupied by them in the former experiment involving the medulla ('25a). The difference lies in the fact that in one case the transplanted segments are preceded by an extraneous medulla, whereas in the other, they are preceded by the normal anterior three segments of the cord.

The results have shown that the anterior end of the cord (first and second segments) when moved caudally in the embryo does not decrease in cellular proliferation to such an extent as was found in the case of the spinal cord reversal experiments involving the anterior limb region ('23b). The transplanted segments approach their typical size and normal cellular proliferation in the more caudal position. The proliferation of cells in the transplanted segments was found to take place more extensively in the dorsal than in the ventral regions (cf. figs. 7 and 8). Moreover, the cellular proliferation in the intact spinal segments cephalad to the transplanted ones was found to be markedly increased, particularly throughout the dorsal regions, which are predominately sensory, and to such an extent that the shapes of these anterior intact segments simulated that of the caudal end of a normal medulla. The striking change in the difference between proliferation throughout the dorsal and the ventral regions of the transplanted segments as compared with the normal, suggests that in these segments capacity for self-differentiation is much higher in the dorsal portion of this region of the cord.

Cellular hyperplasia throughout the dorsal region of the intact anterior segments of the cord under the experimental conditions points to a stimulative influ-

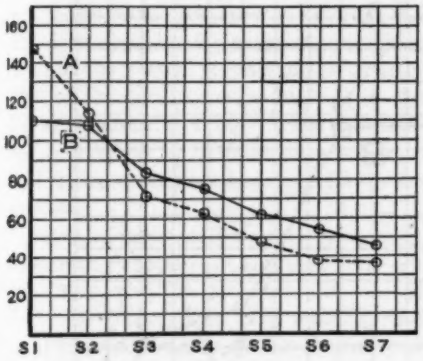


FIG. 7. Curve showing extent of cellular proliferation throughout the dorsal half (curve A) and the ventral half (curve B) of the spinal cord from the level of the first spinal nerve to the level of the seventh in a normal *Amblystoma* larva. Abscissae represent respective spinal nerve levels (1 to 7). Ordinates represent the mean number of cells counted in ten alternate sections through each nerve level. For further explanation v. Detwiler '25d.

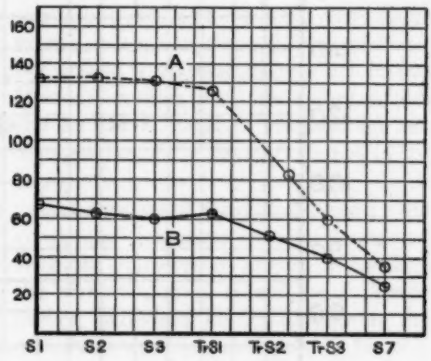


FIG. 8. Curve showing extent of cellular proliferation throughout the dorsal half (curve A) and ventral half (curve B) of the spinal cord from the first to seventh nerve levels in experimental case $TrSC_{23}$. The fourth, fifth, and sixth segments of the spinal cord were replaced by a unit of cord comprising the first three segments from another embryo. Abscissae indicate respective spinal nerve levels (1 to 7). Ordinates indicate mean number of cells counted in ten alternate sections through each nerve level.

ence exerted upon these regions by the transplanted segments lying caudal to them. This influence is seen to be effective only throughout the dorsal regions.

It would appear from the character of the results that there exists in the dorsal region of the anterior segments of the cord, particularly in the first and second segments, sensory centers inherently of strong sensory pathways (spinobulbar) are developed cephalad into the brain.

If these results are viewed from the standpoint of the effects brought about through the influence of growing axones, it would seem that there are two main influences affecting the final proliferation in the anterior segments of the cord. These seem to show a correspondence with

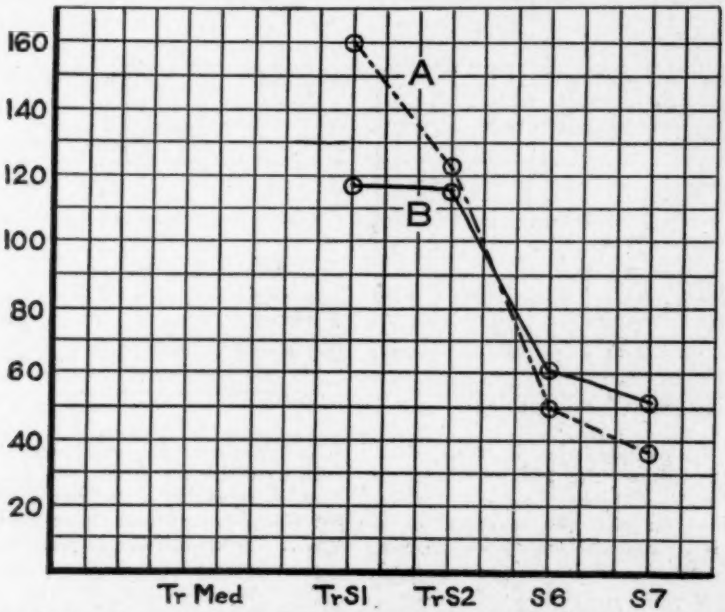


FIG. 9. GRAPH SHOWING EXTENT OF CELLULAR PROLIFERATION IN THE DORSAL HALF (CURVE A) AND THE VENTRAL HALF (CURVE B) OF THE SPINAL CORD IN EXPERIMENTAL CASE TrBrSCl

First five segments of the cord excised and replaced by the caudal end of an extraneous medulla and the first two spinal segments from another embryo. The transplanted first and second spinal segments occupy the position of the normal fourth and fifth respectively. Abscissae and ordinates as in figure 7.

high capacity for proliferation and that a strong influence passes out from this center—particularly in a cephalad direction. The question has been raised previously ('25d) whether or not this influence may be associated with the extent to which ascending tracts arise in this region, for this region is one through which the direction in which the principal pathways are growing. When the grafted anterior segments of the cord are preceded by an extraneous medulla, proliferation in the grafted segments is increased, but the relation between dorsal and ventral proliferation is essentially typical (cf. figs. 7 and 9). On the other hand, when

the anterior segments are transplanted to these same levels, but with the first three segments left intact, proliferation in the dorsal regions of the transplanted segments is disproportionately high. The same is true for the intact anterior segments (figs. 7 and 8). The fact that a normal dorso-ventral relationship results when the grafted spinal segments are preceded by an extra medulla, argues for a bulbar influence on these segments under normal conditions which is stronger ventrally than dorsally. Certainly the inherent capacity for extensive proliferation in the ventral regions of the anterior end of the cord is not as high as in the dorsal regions and only when preceded by an extraneous medulla does this ventral region proliferate in proportion to the dorsal.

How far these results can be brought in line with the action of gradients I am not yet in a position to state. If viewed from this standpoint, they indicate that in the sensory region of the anterior end of the cord, there exists a center of dominance with a physiological influence passing cephalad as evidenced by the cellular hyperplasias occurring throughout the sensory regions lying in front of the graft.

Although numerous agencies are at work in the embryological development of the nervous system (Herrick, '25), there appears to be no doubt from experimental evidence that there is a stimulative influence resulting from the growth of nerve fibers into a field in the process of differentiation.

In addition to the indications brought out in my own experiments, the work of Burr ('20, '23) offers corroboration in this connection. In his earlier work on the removal of the nasal placodes in *Amblystoma* embryos, Burr ('16a) found that the corresponding cerebral hemisphere failed to complete its development. Up to the time when the nasal organ begins to function, the hemisphere on the operated side was as well developed as on the normal side. Since it failed to complete its development after that period, Burr concluded that the hemisphere receives a stimulation from the functional activity of the endorgan (nasal placode) which affects its complete development. In this connection he cited the results of Braus ('06) on the forelimb rudiments of *Bombinator*. Burr ('16b) found further that regeneration of the hemisphere failed in the absence of the olfactory placode, but ensued if the placode was left intact. Here it is evident that some influence from the peripheral organ is essential for the regeneration of the olfactory centers in the brain.

In a later work Burr ('20) came to the conclusion that some stimulus associated with the ingrowth of axones rather than the transmission of stimuli over already formed pathways influences cellular development in the hemisphere. In this connection Burr performed two series of experiments. In the first, the cerebral hemisphere with the adjacent olfactory placode was transplanted to a region just caudal to the right anterior limb and buried beneath the skin. In the second series, the same structures were transplanted to the same region but in this case the olfactory epithelium was healed into the skin and exposed to the exterior as under normal conditions, whereas the telencephalon was deeply buried. Burr found that the telencephalon was as completely organized in one type of experiment as in the other, indicating, therefore, no influence of a functionating end-organ on cellular production in the telencephalon. From his two types of experiments he concluded that the ingrowth of peripheral axones into the wall of the hemisphere was the important agent

influencing cellular proliferation in the hemisphere and not the functional activity of the end-organ. He strengthens his point of view by the observations that the ventral portion of the hemisphere which, by reason of the operation, is cut off from normal ascending fibers is much less differentiated whereas the dorsal portion, the function of which is entirely olfactory, is practically completely developed with the exception of the tracts connecting it with the rest of the brain. Burr says (page 165):

> . . . . Hence it is reasonable to suppose that a part of the differentiation of the nucleus medialis septi and the primordium hippocampi is due to the ingrowth of centripetal fibers.

He says further

> . . . . It is evident then that the factor which produces the second phase of nervous development in the telencephalon is not the functional activity of the nasal epithelium transmitted through the olfactory nerve, but rather the stimulus afforded by the actual ingrowth of neurones into the wall of the hemisphere.

It would be interesting, however, in connection with Burr's experiments to test out whether the olfactory epithelium which was exposed to the surface (second type of experiment) was actually capable of function, and, further, it would be important to know how far proliferation in the olfactory portion of a grafted hemisphere would proceed in the entire absence of an olfactory placode.

The part played by fiber ingrowth in cellular production receives more substantial confirmation in Burr's later experiments ('23) in which he transplanted an accessory nasal placode adjacent to the normal. Here he found that connections were established with the hemisphere and that the augmentation of olfactory fibers thus entering the hemisphere brought about a cellular hyperplasia in the olfactory territory of the same.

In an analysis of Burr's work, Herrick ('25) says

> It may be inferred that the developing olfactory epithelium produces some soluble substance (hormone) which diffuses through the tissues and activates the cells of the adjacent brain wall to more rapid proliferation, or that differences in bioelectrical potentials in the ingrowing olfactory organ can cut across the very short distance between this organ and the brain and so stimulate growth in the latter, or that the permeability of the tissue has been modified in some other way.

Herrick (*op. cit.*) brings out the point that this influence which is exercised upon the developing brain by the rapidly growing peripheral sensory surface may be of much more generalized nature physiologically than a true nervous impulse and indeed may be some phase of the general physiological gradients or some allied kind of process, as he terms it.

Coghill ('24b) has shown that there is a correlation between neuroblastic proliferation in certain regions and axonal ingrowth into the same, but calls attention to the point that, since high rates of proliferation are complete before axonal ingrowth in that region has occurred, the ingrowth of the axones is not the cause of proliferation. His figure 6, page 85, shows that the great increase in neuroblastic proliferation (285.4 per cent) in the cerebrum of *Amblystoma* between the coil stage and the early swimming stage is simultaneous with the ingrowth of the olfactory and the optic nerves. In regard to this point he says, "It is conceivable that the accelerated differentiation in the cerebrum stimulates the ingrowth of the olfactory and optic fibers." He also calls attention to a correlation of the rapid differentiation of neuroblasts in the rhombencephalon at early periods with the ingrowth of the nerve roots.

It is clear from Coghill's work that there are factors other than the growth of nerve

roots which activate proliferation of neuroblasts in the early stages. Nevertheless, there seems to be abundant experimental evidence to suggest that in later stages, at least, cellular proliferation becomes more clearly correlated with fiber invasion, and in fact, may be primarily under the influence of this agent.

In some recent experiments on embryonic eye transplantation (May and Detwiler, '25) we have obtained results which are of especial interest in this connection. The eye was transplanted into a wound resulting from excision of the otic placode (fig. 19). Incidently the olfactory placode was grafted along with the eye. Of 15 cases examined microscopically, the optic nerve was found to enter the IX-X ganglion in 4 cases, the unremoved portion of the VII-VIII ganglion in 3 cases, and in 1 case it entered the medulla. The ingrowth of the axones into these nerve centers brought about a hyperplasia ranging from 22 to 52 per cent of the normal.

The exact manner in which axones growing into a region undergoing differentiation brings about increased cellular production is difficult of analysis. There seems to be at present no valid objection to interpreting the results in line with Cajal's neurotropism theory. Coghill ('24a) brings out the fact that nerve cells grow while they function and suggests that the power of one neurone to activate growth processes in another has origin in the growth phase rather than in the conduction phase of its metabolism. He cites in this connection the experiments of Harrison ('04b) in which he narcotized frog embryos in a 0.02 to 0.03 per cent solution of chloretone during the period when the early nervous pattern is being laid down and found that, upon removal, at a period five days later, they were able to complete normal swimming movements in the course of about five minutes. Certainly this type of experiment argues for an influence other than that brought about through nervous conduction. Recent unpublished experiments by Matthews in the Harvard laboratory show that embryos narcotized for a period of fifteen days undergo a greatly retarded differentiation but that complete recovery of normal reflexes is realized in some cases, although the recovery period is considerably longer than when narcotized for shorter periods of time. It would appear from the character of the results that the delay in recovery results from the prolonged lowering of the growth potentials, though there is, as yet, no quantitive data in this connection.

The action of sensory fibers in bringing about the formation of sensory elements with which they are later in intimate contact, either through contiguity or continuity has been observed in normal development and also in regeneration. Szymonowicz ('95, '96) stated that both in the case of the corpuscles of Merkel and in the corpuscles of Grandy and Herbst, the differentiation of the sense organ is dependent upon the nerve. In the taste buds of fishes (Landacre, '07) and in those of man (Marchand, '02), it appears that the gustatory nerves are the elements which cause differentiation of taste cells. Boeke ('17), Olmstead ('20), and May ('25) found that it is the regenerating sensory nerves which bring about the formation of the new taste cells, following experimental degeneration of the taste buds.

All of these observations may be indicative of the presence of some stimulating substance (secretion) given off by the regenerating nerve which activates the formation of a new taste bud, and it may be that the sensory hyperplasias which have been related to the influence of in-

growing neurones, may be brought about in the same way.

How far hormone action or some allied activity is effectual in these processes it is, of course, difficult to determine.

SELECTIVITY OF NEURONES

Observations by Kappers ('17, '21, '22), Child ('21), Bok ('15), Ingvar ('20) and others offer grounds for theories of the development of neurone pattern and selectivity within the nervous system on the basis of biolectric potentials within the organism, yet when we consider the question of cellular proliferation, peripheral selectivity of axones and other allied phenomena, many interpretations seem difficult unless it is assumed that more or less specific substances (hormones) which exert an attractive influence, are at work. Child ('21) in his discussion of neurone pattern says that in the connection of the nerves with the peripheral territory, chemotaxis may play a part. Herrick ('25) also considers this a real factor in nervous differentiation although he points out that this action is not very specific as is readily indicated by the fact that nerves will grow and effect functional connections in most atypical places. In this connection he cites Burr's ('20) experiments on transplanted olfactory placodes, Stone's ('24) experiments on the transplantation of other cranial placodes, and my own observations on the spinal nerves of *Amblystoma* in effecting connections with transplanted limbs. In the limb experiments it was found that brachial nerves of *Amblystoma* larvae would grow considerable distances out of their normal course to effect functional connection with the grafted appendage ('20b, '22), yet when the limb was grafted at too great a distance, spinal nerves which normally never enter a limb, produced the brachial plexus, in spite of the so-called "preference" of the brachial nerves for their own end-organ. In none of the limb experiments has there ever been any evidence of an absolute muscle-neurone specificity. The same is brought out in the experiments of Harrison ('07a), Braus ('05) and also more recently by Weiss ('23b).

In some recent limb experiments (Detwiler, '25b) the right anterior limb of *Amblystoma* was transplanted the distance of four body segments caudal to the normal situation, under conditions allowing for the regeneration of an appendage in the normal position (figs. 16, 17). This procedure was adopted with the view of bringing about limb development in both heterotopic and orthotopic posi-

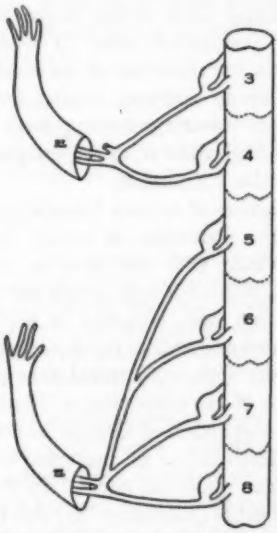


FIG. 10. A DIAGRAMMATIC PLAN OF THE SEGMENTAL NERVE CONTRIBUTION TO THE TRANSPLANTED LIMB (TL) AND THE REGENERATED LIMB (RL) IN CASE AS413

Right anterior limb grafted to position four body segments caudal to the normal. Regeneration of limb in orthotopic position. The normal brachial nerves arise from the third, fourth, and fifth segments of the cord.

tions, and in such a way that initial limb development in the heterotopic position might be advanced over that in the orthotopic position. In many such cases with two anterior limbs, the normal brachial nerves (third, fourth and fifth) were distributed to both appendages although such distribution did not always involve any peripheral communication of the nerves (fig. 10). In several cases, the fifth nerve, which typically supplies the normal limb, was found to grow caudally a considerable distance and supply a large portion of the musculature of the grafted limb. Weiss ('23a, '23b, '23c, '24) recently reported interesting grafting experiments upon differentiated limbs of salamander larvae. In cases where a forelimb was placed near an intact hind limb, the hind limb nerves which were severed in the operation became redistributed to both limbs. In one such case complete innervation of the grafted limb originated from branches of the third lumbar nerve, which normally innervates only the adductors of the femur and the flexors of the knee. Analogous movements of the homologous muscles which were observed in both the experiments of Weiss and myself cannot be correlated, therefore, with specific nerve regeneration to homologous muscles. The acquisition of nerves by the grafted limbs in both types of experiments tends to point towards a similarity between the attractive influence exerted by degenerating nervous tissue in the regenerating nerves such as Weiss observed, and that exerted by the differentiating limb rudiment on outgrowing nerves as was found in my own experiments. It is obvious from the latter experiments that the transplanted limb exerts a stronger influence on the fifth nerve (caudal of the brachial nerves) than does the regenerating limb, otherwise the fifth nerve should supply the orthotopic extremity just as it does under normal conditions. When the normal limb rudiment is not removed and an additional limb rudiment is placed three or four segments caudal to it, the latter is never supplied by the fifth nerve.

It is difficult to analyze the exact nature of this attraction. The differentiating limb rudiment must be regarded as a region of high physiological activity. According to Child ('21) such regions are electropositive with respect to their surroundings. Such being the case, it is conceivable that the axones coming into the general vicinity of the differentiating limb are attracted by the electric field, and electrical polarization may be brought about much after the same fashion as obtains in the central nervous system according to Kappers ('17, '21) or Child ('21). This seems to be applicable to any nerve which develops in the vicinity of the electric field. It may be exemplified by the fact that limbs placed in a position four segments caudal to the normal may receive nerves from the fourth to the eighth segments inclusive, but "preference" seems to be given to the fifth and sixth, presumably because the outgrowth of the nerves from these levels of the cord are in advance of those caudal to them. The only reason offered in explanation of the failure of the third and fourth nerves to grow caudally to the transplanted limb is because the point of high physiological activity (differentiating transplanted limb rudiment) is too far removed to be effective on such a cephalic level.

In the cases with a heterotopic limb placed four segments caudal to the normal and a regenerating limb in the orthotopic position, we have two centers of high physiological activity, but the growth activity of the grafted limb rudiment is exerted earlier than that of the regenerat-

ing one by reason of the initial delay resulting from reorganization in the latter.

Kappers ('21) suggests the probability that in embryos, the proliferation of muscle has the same influence as functionating adult tissue, and that this proliferating tissue may thus activate irradiations of nervous currents from the spinal cord. He makes use of the observations of Herrick and Coghill ('15) on the development of reflex mechanisms in *Amblystoma*, and suggests that the initial contraction of the myotomes sets free action currents already present in the longitudinal tracts of the central nervous system, as was evidenced by the fact that the primary root fibers originate as collaterals from these longitudinal tracts. (Herrick and Coghill, op. cit., fig. 3.)

Bok ('17) has pointed out that the connection between certain muscles and sometimes widely distant places of the central nervous system has to be explained by the fact that the contraction of muscle (which precedes the formation of nerve roots) exerts a trophic action upon the central fibers.

Further evidence of the attraction of differentiating muscle on nerve is shown by the experiments of Hoadley ('25), who transplanted pieces of embryonic chick mesencephalon and somitic tissue to the chorio-allantoic membrane. He found that the nerve fibers which normally are never related to muscle (visual correlation fibers) will grow out from the mesencephalon into the differentiating muscle. Since these fibers will also penetrate into other tissues, Hoadley says that the attraction must be of a very general nature, and in his analysis of the situation is inclined to view the attraction as a dynamic process (galvanic) rather than one of chemotaxis.

Although bio-electric conditions may be responsible for the general ingrowth of axones into a field of rapid proliferation by reason of the high physiological activity set up—hence an electrical polarization (Child, '21), it does not appear to the writer that in the present state of our knowledge we can account for the peripheral selectivity of nerve with muscle without assuming that the muscle or groups of muscle produce substances (hormones?) at particular periods of their differentiation, which act chemotactically upon nerve fibers which are growing into their general vicinity.

In our eye transplantation experiments (May and Detwiler, '25) we found that, in general, there was a shifting of the ganglion towards the entering optic nerve from the transplanted eye. In one case where the transplanted optic nerve entered the medulla, there was a shifting of the nerve cells from their normal position into the white matter at the point of entrance of the nerve. We have already discussed this reaction and similar ones in the light of the theory of neurotropism of Cajal, and of Kappers' neurobiotaxis theory. We also raised the question as to whether or not the cell migration towards

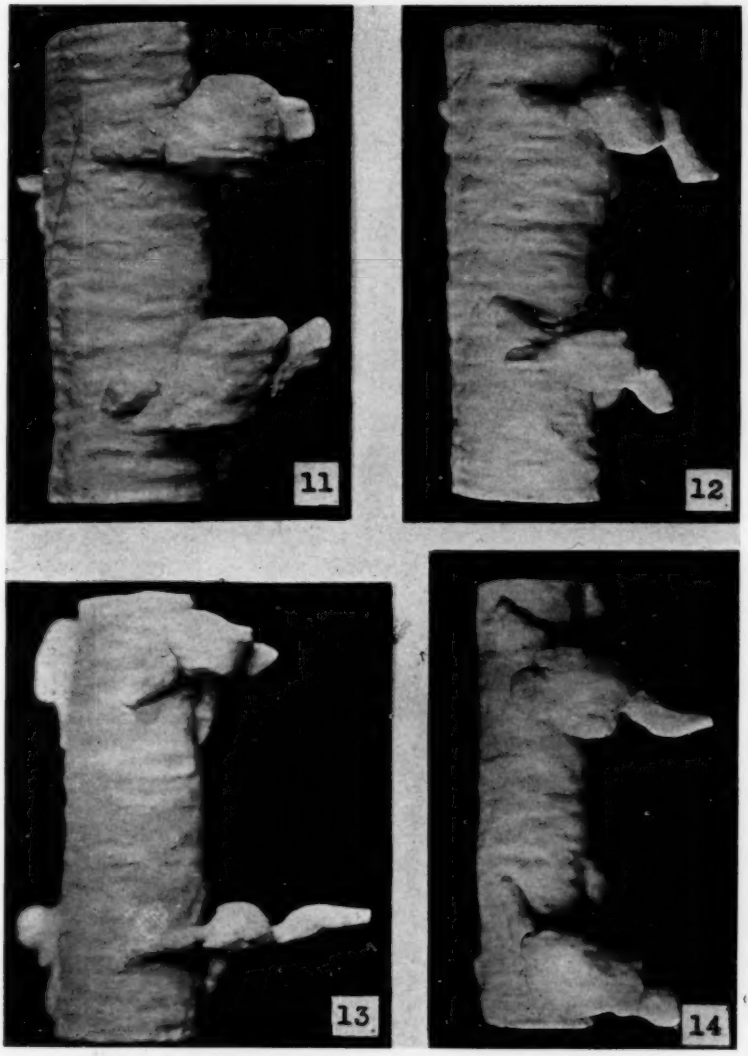
PLATE 1

FIG. 11. Photograph of reconstruction models of the left third and fourth spinal ganglia which are in connection with the normal intact anterior limb. × 50. Case AS_{428}.

FIG. 12. Photograph of reconstruction models of the right third and fourth spinal ganglia, showing reduction in size resulting from excision of the limb with which they are normally connected. × 50.

FIG. 13. Photograph of reconstruction models of the normal left sixth and seventh spinal ganglia which normally have no connection with a limb. × 50.

FIG. 14. Photograph of reconstruction models of enlarged right sixth and seventh spinal ganglia supplying afferent nerves to a transplanted limb. × 50.


11
12
13
14

the entering optic nerve might be an ontogenetic example of Kappers' phylogenetic theory. Further experiments along this line are in progress.

REGENERATION

The agencies which are operative in reparation of the central nervous system of amphibian larvae following experimental injury have been studied in an experimental way by a number of investigators. Hooker ('15) completely severed the spinal cord of frog embryos at the stage of closed neural folds with a view of analyzing the processes leading to its reunion. He found that the spinal cord underwent complete regeneration and that the process of reunion is affected primarily by the development of nerve fibers which bridge the gap and establish physiological continuity. He calls attention to similar observations made by Brown-Séquard ('49) and by Harrison ('10b). Whereas Hooker found that both pieces of the sectioned cord played a rôle in the reunion process, Masius and Van Lair ('69) claimed that the caudal stump played no part in the regeneration of the cord in adult frogs.

Hooker (*op. cit.*) found that when the cut ends of the spinal cord were brought into contact with each other, healing per primam resulted. When the wound surfaces were somewhat separated, reunion was established by a definite sequence of events. Using his wording these are: (*a*) the development of nerve fibers from the motor cells of each segment of the cord, (*b*) the growth of sensory axones from the cut surface of the posterior stump, (*c*) the outgrowth of fibers from the epithelial cells of the canalis centralis of either end of the cord, (*d*) the wandering of neuroblasts into the fibrous net between the cut ends from both stumps, and (*e*) the elongation of both ends of the spinal cord towards each other by the proliferation of epithelial cells of the canalis centralis and the consequent lengthening of the canal.

Hooker also showed that the elements entering into the regenerated portion of the cord are derived entirely from the original cord, and that neither connective tissue nor epithelium take any part in the process.

In his second paper, Hooker ('17) reports the results of reversing end-for-end a portion of the cord on the healing of the cord wounds, the polarity of the elements of the cord, and upon the behavior of the larvae. He found as did Spemann ('12) that the reversed piece retained its original anatomical polarity. Under simple section of the cord the reunion is established first by the caudal growth of the motor processes from the cephalic stump, which in turn is followed by the cephalic growth of sensory processes. In the embryo with a reversed middle piece, following double section of the cord in the cervical region, the normal relationship between the direction of the growth of all the processes and the antero-posterior axis of the body has been completely upset so that the nerves which were originally descending processes grew in an ascending direction and vice versa. Also the reversal brings together at the cephalic wound surface a series of nerve fibers growing in the opposite direction which are all descending processes, and, at the caudal wound a number of fibers growing in both directions which are ascending processes. Under these conditions there was present a marked tendency on the part of the nerve fibers to avoid entering the opposite wound surface. In spite of this "antagonism" between "like" surfaces, fibers did bridge the gap in many cases and reestablish continuity.

Although the neurones begin their

development in the normal orientation to the reversed piece, the direction of the transmission of stimuli is reversed as evidenced by the normal reflex behavior of the larvae. Regarding this point Hooker (op. cit., page 445) remarks

> . . . . It is of course doubtful whether there is any real specificity of ascending and descending processes and the physiological results obtained certainly demonstrate that a considerable degree of adaptation has taken place here, in that the descending processes must certainly function as ascending processes and vice versa. In this sense, therefore, we must conclude that there is a reversal in the polarity of the elements contained within the reversed piece of the spinal cord, though whether this reversal in polarity includes anatomical reversal of the cells themselves is very doubtful. It is much more probable that only the direction in which the stimuli travel along the processes is the reverse of its usual course.

Corroboration of such functional reversal as obtained by Hooker has been physiologically brought out in the spinal cord reversal experiments in *Amblystoma* embryos (Detwiler, '23b).

In a recent paper Hooker ('25) has described the processes which establish anatomical continuity in transected cords of frog tradpoles, and his paper gives a résumé of work done by other investigators in this field. His observations show that the structural restoration of the severed spinal cord demands the presence of four types of elements. According to him these are: ependymal cells, neuraxes derived from neuroblasts of the original cord, spongioblasts, and neuroblasts. The principal problem according to him is the supply of spongioblasts and neuroblasts to restore the other elements of the cord. Apparently the necessary neuroblasts and spongioblasts may be derived by the cytomorphosis of cells already present, or by the production of additional elements by proliferation. A shifting of cells and their migration for greater or lesser distances also seems to be necessary.

In addition to showing the cellular elements requisite for regeneration of the cord, and the methods by which the repair processes are carried out, Hooker's ('25) observations also indicate the existence of some directive stimulus on the growth of the neuraxes. The same conclusion is made by de Nó ('21) who speaks of it as a neurotropism.

Other evidence of attraction in the growth and restitution processes following cord transection are brought out in the experiments of Wieman ('22). Wieman rotated pieces of spinal cord at ninety degrees to the antero-posterior axis and

---

PLATE 2

FIG. 15. Photograph of Amblystoma larva showing right anterior limb transplanted the distance of four body segments caudal to the normal position. Fifty days after operation. × 2.

FIG. 16. Photograph of *Amblystoma* larva (fifty-three days after operation), showing right anterior limb transplanted (with inverted orientation) the distance of four body segments caudal to the normal position. Regeneration of limb in orthotopic position. × 2.

FIG. 17. Photograph of *Amblystoma* larva (fifty days after operation) with right anterior limb rudiment transplanted the distance of three body segments caudal to the normal position. Transplanted extremity completely reduplicated. Regeneration of limb in orthotopic position. × 2.

FIG. 18. Photograph of *Amblystoma* larva (seventy days after operation) in which the anterior limb region of the spinal cord (third, fourth, and fifth segments) was excised and replaced by a more caudal unit of cord (seventh, eighth and ninth segments) from another embryo. × 2.2.

FIG. 19. Photograph of *Amblystoma* larva showing transplanted eye in position of the ear. × 4.

FIG. 20. Photograph of *Amblystoma* larva Tr5c 30 in which the third, fourth, and fifth spinal segments have been replaced by a unit of cord comprising the first, second and third segments from another embryo. × 2.4.


15


16


17


18


19


20

purposefully interposed somitic tissue between the intact stumps and the rotated piece. In this way healing per primam was prevented, and the reunion was established first by the caudal growth of descending processes which was followed by the cephalic growth of ascending processes as observed by Hooker ('17). He found that the descending processes always pierce the rotated piece through its originally cephalic end, and the ascending ones always enter the originally caudal surface, yet the chances for the opposite course are equally advantageous as far as distance is concerned. Wieman corroborates Hooker in that the descending tracts are reëstablished before the ascending ones. In fact Wieman states that the development of ascending tracts depends upon a prior formation of descending ones through the operated region. This is strongly suggested by the greater frequency of fusion between the rotated piece and the stump found to obtain in higher levels of the cord as compared with the more caudal levels. The relation found to occur between the time of motor and sensory reunion bear out in an experimental way Coghill's observations on the development of reflex arcs of the cord.

Wieman ('25) found, that when the sectioned piece was oriented at 135 degrees to the normal, some cases healed per primam and a complete reversal of functional polarity ensued. In others, the isolation of the transplanted piece was maintained for a long enough time to allow the inherent polarity to come to full expression. In such cases descending connections failed. Wieman believes that this is due to the fact that the metabolic gradient set up at an angle of 135 degrees to the principal gradient inhibits the formation of descending tracts to a greater extent than when the gradient of the transplanted piece forms an angle of 90 degrees.

Wieman's results show that nervous reunion between the transplanted neural tube and stumps depends primarily upon the development of descending outgrowths from the anterior neural stump.

Since both Hooker and Wieman find that the first repair process in cases not healing per primam is the outgrowth of neuraxes, and that this is followed by proliferation of cells in the original cord (Hooker, '25), it may be that this proliferation is set up under a stimulative influence of fiber invasion. If this should actually prove to be the case, it would place regeneration processes at least in part upon the same basis as those found in normal morphogenesis.

Another subject of general interest to the students of the nervous system pertains to the influence of the developing nervous system upon the morphogenesis and regeneration in other systems. This matter is under experimental investigation and constitutes a subject which space will not permit of discussion in this paper. Recent researches by Weiss ('25) and by Hamburger ('25) show a controlling influence of the nervous system upon the normal development and regeneration of the extremities in amphibians. Their papers contain discussions of the researches of others in this field, as well as valuable bibliographies.

It has not been the purpose in this communication to review the entire subject of morphogenesis in the nervous system, but merely to point out the trend of certain lines of experimentation in this field, and the results which they have so far achieved. It is hoped that continued work along such and other lines may contribute to clearer interpretations of the perplexing problems of morphogenesis and to our knowledge of structure-function correlations in the nervous system.

LITERATURE LIST

Banchi, H. 1906. Sullo sviluppo bei nervi periferici in maniera independente dal sistema nervosa centrale. Anat. Anz., Bd. 28.

Bellamy, A. W. 1919. Differential susceptibility as a basis for modification and control of early development in the frog. Biol. Bull., vol. 37.

Bokke, J. 1917. Studien zur Nervenregeneration. II. Die Regeneration nach Vereinigung ungleichartigen Nervenstücke (heterogene Regeneration) und die Funktion der Augenmuskel- und Zungennerven. Die allgemeinen Gesetze der Nervenregeneration. Verhand. d. Konink. Akad. v. Wetensch. t Amsterdam, D. 19.

Bok, S. T. 1915. Die Entwicklung der Hirnnerven und ihrer zentralen Bahnen. Die stimulogene Fibrillation. Folia Neurobiolog., Bd. 9.

1917. The development of reflexes and reflex tracts. 1. The reflex circle. Psychiat. en Neurolog. Bladen, Amsterdam, vol. 21.

Braus, H. 1904. Einige Ergebnisse der Transplantation von Organanlagen bei *Bombinator* larven. Verh. Anat. Ges., Bd. 18. Vers. Jena.

1905. Experimentelle Beiträge zur Frage nach der Entwickelung peripherer Nerven. Anat. Anz. Bd. 26.

1906. Vordere Extremität und Operculum bei *Bombinator* larven. Morph. Jahr., Bd. 35.

Brown-Séquard, C. E. 1849. Exposé sur la curabilité des plaies de la moelle épinière. Compt. rend. Soc. Biol., T. 1.

Burr, H. S. 1916a. The effect of the removal of the nasal placodes on *Amblystoma* embryos. Jour. Exp. Zool., vol. 20.

1916b. Regeneration in the brain of *Amblystoma*. Jour. Comp. Neur., vol. 26.

1920. The transplantation of the cerebral hemispheres of *Amblystoma*. Jour. Exp. Zool., vol. 30.

1923. Experimental hyperplasia of the cerebral hemispheres in *Amblystoma*. Anat. Rec., vol. 25.

Burrows, M. T. 1911. The growth of tissues of the chick embryo outside the animal body with special reference to the nervous system. Jour. Exp. Zool., vol. 10.

Cajal, S. Ramón y. 1890. A quelle époque apparaissent les expansions des cellules nerveuses de la moelle épinère des poulets. Anat. Anz., Bd. 5.

1892. La rétine des vertébrés. La Cellule. T. 9.

1894. Les nouvelles idées sur la structure du système nerveux chez l'homme et chez les vertébrés. Paris.

1906. Genesis de las fibras nerviosas del embrion y observaciones contrarias a la teoria catenaria. Trab. Lab. Invest. Biol. Univ. Madrid, T. 4.

Child, C. M. 1921. The Origin and Development of the Nervous System. Univ. Chicago Press. Chicago.

1924. Physiological Foundations of Animal Behavior. Henry Holt and Co., New York.

Coghill, G. E. 1923. The growth of neuroblasts in relation to physiological and differential rates of metabolism. Abstracts of papers, Amer. Assoc. Anatomists, Chicago.

1924a. Correlated anatomical and physiological studies of the growth of the nervous system in Amphibia. III. The floor plate of *Amblystoma*. Jour. Comp. Neur., vol. 37.

1924b. Correlated anatomical and physiological studies of the growth of the nervous system in Amphibia. IV. Rates of proliferation and differentiation in the central nervous system of *Amblystoma*. Jour. Compt. Neur., vol. 37.

Detwiler, S. R. 1920a. On the hyperplasia of nerve centers resulting from excessive peripheral loading. Proc. Nat. Acad. Sci., vol. 6.

1920b. Experiments on the transplantation of limbs in *Amblystoma*. The formation of nerve plexuses and the function of the limbs. Jour. Exp. Zool., vol. 31.

1922. Experiments on the transplantation of limbs in *Amblystoma*. Further observations on peripheral nerve connections. Jour. Exp Zool., vol. 35.

1923a. Experiments on the transplantation of the spinal cord in *Amblystoma* and their bearing upon the stimuli involved in the differentiation of nerve cells. Jour. Exp. Zool., vol. 37.

1923b. Experiments on the reversal of the spinal cord in *Amblystoma* embryos at the level of the anterior limb. Jour. Exp. Zool., vol. 38.

1924. Further observations on proliferation of nerve cells in grafted units of spinal cord. Anat. Record, vol. 27.

1925a. The results of substituting an extraneous medulla for the cephalic end of the embryonic spinal cord in *Amblystoma*. Jour. Exp. Zool., vol. 41.

1925b. Coordinated movements in supernumerary transplanted limbs. Jour. Comp. Neur., vol. 38.

1925c. Spinal cord injury and nerve cell proliferation in the embryo. Anat. Record, vol. 30, 39.

1925d. An experimental study of cellular proliferation in the anterior end of the spinal cord of *Amblystoma*. Jour. Exp. Zool., vol. 42.

DETWILER, S. R., AND LEWIS, RUTH. 1925. Size changes in primary brachial motor neurones following limb excision in *Amblystoma*. Jour. Comp. Neur., vol. 39.

DE NÓ, R. LORENTE. 1921. La regeneración de la medula espinal en las larvas de batracio. Trabajos. T. 19.

DÜRKEN, B. 1911. Über frühzeitige Extirpation von Extremitatenanlagen beim Frosch. Zeit. f. wiss. Zool., Bd. 99.

FOREL, AUGUST 1887. Einige hirnanatomische Betrachtungen und Ergebnisse. Arch. für Psychiatrie. Bd. 18.

GEMELLI, F. A. 1906. Ricerche sperimentali sullo sviluppo dei nervi degli arti pelvici di *Bufo vulgaris* innestati in sede anomala. Revista di Pathologia Nervosa e Mentale. Anno. 11.

HAMBURGER, VICTOR 1925. Über den Einfluss des Nervensystems auf die Entwicklung der Extremitäten von *Rana fusca*. Arch. f. mikros. Anat. u. Entw.- mech., Bd. 105.

HARRISON, R. G. 1904a. Neue Versuche und Beobachtungen über die Entwicklung der peripheren Nerven der Wiebeltiere. Sitz.-Ber. Niederrh. Ges. Natur. u. Heilkunde. Bonn.

1904b. An experimental study of the relations of the nervous system to the developing musculature in the embryo of the frog. Am. Jour. Anat., vol. 3.

1906. Further experiments on the development of peripheral nerves. Amer. Jour. Anat., vol. 5.

1907a. Experiments in transplanting limbs and their bearing upon the problems of the development of nerves. Jour. Exp. Zool., vol. 4.

1907b. Observations on the living developing nerve fiber. Anat. Record, vol. 1.

1910a. The outgrowth of the nerve fiber as a mode of protoplasmic movement. Jour. Exp. Zool., vol. 9.

1910b. The development of peripheral nerves in altered surroundings. Arch. f. Entw.-mech., Bd. 30.

HENSEN, V. 1864. Über die Entwickelung des Gewebes und der Nerven im Schwanze der Froschlarve. Virchow's Archiv. Bd. 31.

HERRICK, C. J. 1914. The medulla oblongata of larval *Amblystoma*. Jour. Comp. Neur., vol. 24.

1922. Some factors in the development of the Amphibian nervous system. Anat. Rec., vol. 23.

1925. Morphogenetic factors in the differentiation of the nervous system. Physiol Reviews, vol. 5.

HERRICK, C. J., AND COGHILL, G. E. 1915. The development of reflex mechanisms in *Amblystoma* embryos. Jour. Comp. Neur., vol. 25.

HIS, W. 1886. Zur Geschichte des menschlichen Rükenmarks und der Nervenwurzeln. Abh. d. math-phys. Kl. d. Kgl. Sachs. Ges. d. wiss., Bd. 13.

1887. Die Entwickelung der ersten Nervenbahnen beim menschlichen Embryo. Arch. f. Anat. u. Physiol. Anat. Abt.

1888. Zur Geschichte ges Gehirns sowie der centralen und peripherischen Nervenbahnen beim menschlichen Embryo. Abh. d. math.-phys. Kl. d. Kgl. Sächs. Ges. d. wiss., Bd. 14.

1890. Histogenese und Zusammenhang der Nervenelemente. Arch. f. Anat. u. Phys., Anat. Abt. Suppl. Bd.

HOADLEY, LEIGH 1925. The differentiation of the isolated chick primordia in chorio-allantoic grafts. III. On the specificity of nerve processes arising from the mesencephalon in grafts. Jour. Exp. Zool., vol. 42.

HOOKER, DAVENPORT 1915. Studies on regeneration in the spinal cord. I. An analysis of the processes leading to its reunion after it has been completely severed in frog embryos at the stage of closed neural folds. Jour. Comp. Neur., vol. 25.

1917. Studies on regeneration in the spinal cord. The effect of reversal of a portion of spinal cord at the stage of closed neural folds on the healing of the cord wounds, on the polarity of the elements of the cord and on the behavior of frog embryos. Jour. Comp. Neur., vol. 27.

1923. The nature of the division of neuroblastic cells in the regenerating spinal cords of Amphibian larvae. Anat. Rec., vol. 24.

1925. Studies on regeneration in the spinal cord. III. Reëstablishment of anatomical and physiological continuity after transection in frog tadpoles. Jour. Comp. Neur., vol. 38.

INGVAR, SVEN 1920. Reaction of cells to the galvanic current in tissue cultures. Proc. Soc. Exp. Biol. and Med., vol. 17.

KAPPERS, C. U. ARIËNS 1917. Further contributions on neurobiotaxis. IX. An attempt to compare the phenomenon of neurobiotaxis with other phenomena of taxis and tropism. The dynamic polarization of the neurone. Jour. Comp. Neur., vol. 27.

1921. On structural laws in the nervous system: The principles of neurobiotaxis. Brain, vol. 44.

1922. Dixième contribution a la théorie de la neurobiotaxis. L'Encephale, i, 1.

LANDACRE, F. L. 1907. On the place of the origin and method of distribution of taste buds in *Amiurus melas*. Jour. Comp. Neur., vol. 17.

v. LENHOSSÉK, M. 1892. Beobachtungen an den Spinalganglien und dem Rückenmark von *Pristiurus* embryonen. Anat. Anz., Bd. 7.

v. Lenhossék, M. 1895. Der feinere Bau des Nervensystems im Lichte neuester Forschungen. Berlin.

1906. Zur Frage nach der Entwicklung der peripheren Nervenfasern. Anat. Anz., Bd. 28.

Lewis, M. R., and W. H. 1911. The cultivation of tissues from chick embryos in solutions of NaCl, $CaCl_2$, KCl, $NaHCO_3$. Anat. Rec., vol. 5.

Marchand, L. 1902. Développment des papilles gustatives chez le foetus humain. Compt. Rend. Soc. Biol. Paris, T. 54.

Masius, V., und Van Lair, C. 1869. Anatomische und functionelle Wiederherstellung des Rückenmarks beim Frosch. (Vorhäufige Mittheilung). Centrlbl. f. d. med. Wissen., 7.

May, R. M. 1925. The relation of nerves to degenerating and regenerating taste buds. Jour. Exp. Zool., vol. 42.

May, R. M., and Detwiler, S. R. 1925. The relation of transplanted eyes to developing nerve centers. Jour. Exp. Zool., vol. 43.

Olmstead, J. M. D. 1920. The nerve as a formative influence in the development of taste buds. Jour. Comp. Neur., vol. 31.

Roux, W. 1885. Einleitung. Archiv. f. Entw.-mech. des Organismen, Bd. 1.

Shorey, M. L. 1909. The effect of the destruction of peripheral areas on the differentiation of neuroblasts. Jour. Exp. Zool., vol. 7.

1911. A study of the differentiation of neuroblasts in artificial culture media. Jour. Exp. Zool., vol. 10.

Stone, L. S. 1924. Experiments on the transplantation of placodes of the cranial ganglia in the amphibian embryo. I. Heterotopic transplantations of the ophthalmic placode upon the head of *Amblystoma punctatum*. Jour. Comp. Neur., vol. 38.

Spemann, H. 1912. Über die Entwicklung ungedrehter Hirnteile bei Amphibienembryonen. Zool., Jahrb., Suppl. Bd. 15.

Sztmonowicz, W. L. 1895. Beiträge zur Kenntniss der Nervenendigungen in Hautgebilden. Arch. f. mikros. Anat., Bd. 45.

1896. Ueber den Bau die Entwicklung der Nervendigungen im Entenschnabel. Arch. f. mikros. Anat., Bd. 48.

Weiss, Paul 1923a. Die Funktion transplantierter Amphibien-extremitäten. II. Kompensatorische Reflexe. Sitzungsber. d. Akad. d. Wiss. Wien. Akad. Anz., Nr. 10, Mitt. 99.

1923b. Die Funktion transplantierter Amphibienextremitäten. III. Histologische Untersuchungen über die Nervenversorgung der Transplantate. Sitzungsber. d. Akad. d. Wiss. Wien. Akad. Anz., Nr. 22–23, Mitt. 80.

1923c. Die Funktion transplantierter Amphibienextremitäten. IV. Theorie: Die Erfolgsorgane als Resonatorensystem. Ibid., Mitt. 101.

1924. Die Funktion transplantierten Amphibienextremitäten. Aufstellung einer Resonanztheorie der motorischen Nerventätigkeit auf Grund abgestimmter Endorgane. Archiv. für mikros. Anat. u. Entw.-Mech., Bd. 102.

1925. Abhängigkeit der Regeneration entwickelter Amphibienextremitäten vom Nervensystem. Archiv. f. mikros. Anat. u. Entw.-Mech., Bd. 104.

Wilman, H. L. 1922. The effect of transplanting a portion of the neural tube of *Amblystoma* to a position at right angles to the normal. Jour. Exp. Zool., vol. 35.

1925. Further observations on the angular transplantations of the neural tube of *Amblystoma*. Jour. Exp. Zool., vol. 41.

A REVIEW OF THE DISCOVERY OF PHOTOPERIODISM: THE INFLUENCE OF THE LENGTH OF DAILY LIGHT PERIODS UPON THE GROWTH OF PLANTS

By K. F. KELLERMAN

Bureau of Plant Industry, United States Department of Agriculture

LIGHT is so obviously important for the well-being of most living things that, as might be expected, there is a wealth of literature describing the results of careful studies of the effect on plants of sunlight and of artificial light, of light of different colors, and of different intensities.

It was not, however, until the painstaking and brilliant researches of Dr. W. W. Garner and his associates that the remarkable significance of changes in the duration of the daily periods of light and darkness was realized. For many plants, perhaps for most plants of the temperate zone, the length of the day—that is, the number of hours and minutes of continuous daylight—is a far more critical factor in determining character of growth than is the intensity of the light. For example, the common blue violet is known as a typical spring flower. In other words, these blue flowers are produced at a time of year when the daylight period is relatively short. Violets may be produced at any season of the year, however, by exposing the plants to the proper number of hours of light (plate 1, A); thus even in midsummer typical blue violets may be secured by placing the plants in an absolutely dark room, and each day bringing them out into the sunlight for a period of about 10 hours. On the other hand, the common rosemallow does not flower unless it is exposed daily to a light period of more than 12 hours. Buckwheat, which is capable of producing flowers throughout the range of a daylight period varying from 5 hours up to 20 hours, and probably even in continuous illumination, is an extreme example of a third type of plants, overlapping but very distinct from the first two.

PHOTOPERIODISM

Garner has coined the word "photoperiodism" to designate these responses to length of day, and for convenience in describing the behavior of different plants has arbitrarily grouped those experimented with up to the present time into (1) short-day, (2) long-day, and (3) everblooming plants.

Using the equatorial length of day of 12 hours as a standard, plants belonging to the first or short-day group are those which can bloom only under a daily light period of 12 hours or less; plants of the second or long-day group can bloom only in a day light length of more than 12 hours; plants of the third group are capable of blooming throughout these ranges. It should be noted that not only botanical species but even horticultural varieties and strains differ markedly as to the particular length of day most favorable for flowering. Furthermore, they also differ widely as to the narrowness of the range

in day length which will permit flowering. Climbing hemp-weed, for example, can flower only between the daylight ranges of 13½ hours to 15½ hours, the most favorable period being approximately between these figures. The Biloxi variety of soybeans, however, which cannot flower in a day length in excess of 13 hours and with an optimum flowering period of approximately 10 hours, cannot be given a sufficiently short light day to entirely suppress flowering and maturity. The continuity of the light period has been assumed in these statements. Alternate exposure to 1-hour periods of light and darkness, although perhaps enabling a plant to grow vigorously, is not equivalent to a 12-hour daily exposure to continuous light; and plants which normally flower actively in the 12-hour daylight period are totally unable to flower under alternate hours of light and darkness, even though they are thus receiving 12 hours of light out of the 24. Curiously enough, however, a single break in an extended daylight period appears to have little if any effect upon the response to the light period measuring from the beginning to the end. A plant, such as the Biloxi soybean (plate 1, B), capable of flowering actively in a 12-hour light period will continue to develop in an apparently identical manner if placed in the dark during 2 hours in the center of each light period so that it is actually receiving but 10 hours of light daily instead of 12. It would thus seem that whatever reactions or processes may be initiated in the plant by the light rays, some time is required to bring about these responses, and that the momentum of the reaction is sufficient to bridge the gap caused by a single short dark period in the middle of the day. It is not possible, as yet, to determine how extensively the light period may be broken before interfering with its controlling effect on the plant; nor is it possible to determine the limits of decreasing intensity of light below which plants do not react. It is noteworthy, however, that supplementing the daily period of sunlight with ordinary incandescent lights of approximately one one-thousandth of the intensity of sunlight is sufficient to bring about the flowering response of long-day plants.

LENGTHENING DAYS WITH ELECTRIC LIGHT

Equipping an ordinary greenhouse with incandescent lights giving an average light intensity of about 4 candlepower at the surface of the soil was found sufficient for bringing many plants into blossom, although this light contains practically no ultra violet rays and is equally lacking in many other rays present in sunlight. Even differences in color of light have been found of relatively little significance in causing or preventing flowering. This weak light used to supplement the short days of the winter season was turned on at sunset and extinguished at midnight in each 24 hours. The short-day plants, such as common cosmos and narrow leaved sunflower (plate 2, A and B) in the artificially-lighted greenhouse were unable to flower but continued vigorous vegetative growth. When grown in a greenhouse receiving only the natural light of the winter day but under the same conditions of temperature and moisture these same species of plants, though very small, flowered promptly. The long-day plants, on the other hand, such as golden rod and Coreopsis (plate 3, A and B) grew but poorly in the greenhouse receiving only winter daylight, while they grew vigorously and produced their flowers in a normal summer manner in the greenhouse where the winter day was artificially lengthened by the electric light. Spinach planted in the naturally lighted greenhouse developed a

typical rosette of leaves throughout the winter, while in the electrically-lighted house it bloomed within 6 weeks of germination. Spinach is mentioned particularly, because hitherto the generally accepted idea of the outgrowing of the rosette stage and sending up blooming stalks of spinach is that it was caused by the advancing temperature of summer.

LOCALIZED FLOWERING RESPONSE

The extremely local character of the flowering response to the daylight period is so remarkable that it deserves special consideration. Using cosmos as the basis of the experiment, young seedlings propagated under long-day conditions were cut back and forced to develop two approximately equal branches. Some time afterward cardboard screens were placed between the two branches so that each branch of the same plant could be given different periods of illumination (plate 4, A). Under these conditions the branch exposed to the short day promptly flowered, matured, and passed into a typical state of decline following maturity, while the branch exposed to the longer illumination period continued the vigorous vegetative type of growth. The lower stem of the plant continued to grow in harmony with the development of the vegetative branch and apparently was unaffected by the mature and dying condition of the branch which had fruited. Elaborating these experiments, specially constructed light-proof boxes were placed about different portions of a well developed cosmos plant. When the upper portion of the plant was exposed to approximately 15 hours of light while the lower portion was given 10 hours of light, flowers promptly developed on the lower portion, while the upper portion remained in vigorous vegetative condition. When both the top and bottom third of the plant were exposed to the short light period and the central portion was exposed to the longer light period, flowering and fruiting on the top and bottom thirds very soon developed while the central portion remained vigorously vegetative (plate 4, B). If carefully handled, these localized responses may be continued for several months. In so far as the development of the flowering habit is concerned, therefore, it seems that each portion of the plant is capable of responding to the stimulus of the light period in much the same manner as if it were a separate plant.

LIGHT AS AN ECOLOGICAL FACTOR

The daylight period exerts a very striking and determinative influence on many phases of growth as well as upon flowering and fruiting but before discussing these more involved problems especial attention should be called to the important action of the light period in differentiating groups of plants by its limiting effect upon their ability to reproduce their species. Other environmental factors, such as temperature, moisture, and character of food supply also exert a selective action, but usually each of these factors may change throughout a rather wide range before the existence of the individual plant is jeopardized or its behavior or type of growth (as distinguished from vigor of growth) is markedly affected. The light period, however, exhibits a diversity of effect upon different kinds of plants of a more far-reaching character. Plants which require a long day to flower and fruit obviously can not maintain themselves in the tropical zone, where the day remains about 12 hours for the entire year, even though high altitude may hold the temperature well below the danger limit for the species. The more extreme type of short-day plants are, of course, equally out of the question for the tropics.

The everblooming groups can follow the weather and the soil and can maintain a foothold wherever these conditions are satisfactory or competition with other plants permit. In so far as records are available, it appears that everblooming plants are prevalent in the tropics, though, in accordance with Garner's theory, a species or variety might be everblooming there and be either everblooming or a spring or a fall flower in the temperate zone. Cold weather may delay growth and consequently delay flowering, and hot weather may hasten it, but if the light period is suitable for a particular plant it will flower as promptly as the climatic conditions permit, while if the light period is not suitable, weather changes cannot cause flowering. The apparently erratic behavior of certain plants brought to new areas of approximately similar climate is probably to be explained largely if not entirely as a length of day response, for even a difference of minutes may cause a change of growth.

VEGETATIVE RESPONSE TO LIGHT PERIODS

To recur to the recent experimental work on the responses of different plants to the length of the daylight period, the changes in the character of vegetative growth are as clear and as striking as is the change from the vegetative to the flowering behavior previously discussed. A common spring radish, the Globe variety, if exposed to a 7-hour day will continually enlarge its bulbous root at least for a year and probably indefinitely (plate 5, **A**). Similarly, the Irish potato, Jerusalem artichoke, and the yam develop tubers only under comparatively short days. Carrots, on the other hand, do not produce a bulbous root under a very short day but under a 14-hour day produce the typical carrot root, well stored with reserve food materials. The silverskin onion produces a typical spring onion when grown under a 10-hour day, a slightly bulbous spring onion under a 13-hour day, and a typical summer onion bulb when grown under a 15-hour day (plate 5, **B**). In general, it appears that plants differ in the light period best suited to the production of reserve food supplies whether these reserves are developed as corms, bulbs, or tubers, or as resting buds. In no case in these experiments has the light period best adapted to formation of bulbs or other reserves of food material coincided with the daylight period best adapted for upward or top growth of the particular plant under consideration. In this particular the depositing of reserve food apparently differs from the flower response for it is not impossible for the daylight period suitable for flowering and for upward or top growth of a plant to coincide or at least to materially overlap. Comparison of Baldwin apple seedlings with seedlings of the box-elder emphasizes a wide difference of the optimum light exposure for upward growth. These apple seedlings grow most vigorously when exposed to approximately 10 hours of light daily and their growth is seriously depressed with extension of the light period to 14 or 15 hours (plate 6, **A**). It is not noticeably affected by a light period even much shorter than 10 hours. The box-elder, however, is scarcely able to grow at all with a day as short as 10 hours and makes its most vigorous growth when exposed to a 14- or 15-hour day (plate 6, **B**).

With these seedling trees, as well as with several other plants, it seems possible not only to depress vegetative growth but actually to carry this depression to the point of killing the plant merely by adjusting the light period to one extremely unfavorable for vegetative growth. As might be expected, there are numerous

species of plants for which no light period has yet been found that will completely suppress vegetative growth unless associated with fruiting and maturity. The classical idea of the normal death of a plant, especially as applied to annuals, is that death should occur following the maturation period which accompanies flowering and fruiting. While there is a very clear correlation existing between the fruiting of annuals and their immediately subsequent death, this is clearly not a necessary sequel. With many annuals, for example, the beggar tick, after flowering and fruiting has begun, a change of the light period to one more suitable for vegetative growth than for flowering will re-establish vigorous vegetative growth which within the limits of these experiments at least may be indefinitely prolonged. The death of annuals following their fruiting therefore would appear to be largely a reaction to an unfavorable length of day for vegetative growth exaggerated by the weakening of the plant through the strain of formation of flowers and development of seed. In both the box-elder and the Baldwin apple seedlings and in the case of the sumac, tulip-poplar, etc. leaf-fall can be brought about by shortening the day regardless of the favorableness of temperature and moisture conditions, while leaf-fall can be completely prevented throughout the winter if the short days of winter are supplemented by the use of electric light (plate 7, **A** and **B**). The distinction between evergreens and deciduous perennials may be therefore an expression of the reaction of these plants to the length of day prevailing in the latitudes in which they naturally occur.

PHYSIOLOGICAL SIGNIFICANCE OF LIGHT AND OTHER FACTORS

It is not the intention of the authors of these experiments to indicate that light is in itself the sole cause of the remarkably diverse growth effects that result from changes in the light period. The length of day reaction is rather to be regarded as one of the most important factors in controlling the normal physiology of growing plants by modifying the function or the activities of the plant cells. Some of the experiments briefly referred to have demonstrated the comparatively slight but consistent changes in acidity of the cell sap in different portions of the plant as a result of the changes of length of day and these changes in acidity are directly correlated with the changes in the growth habits. In the case of short-day plants, for example, abrupt transfer from a long day to a short day causes a sudden and sharp decrease in acidity in the region of most active growth; this usually occurring about 3 to 5 days after the change in light period has been made. This drop in acidity, which is believed to indicate definite transition from the vegetative to the flowering condition, is only temporary and is followed by an equally rapid rise to almost the original level of acidity. The acidity relations resulting from exposure to the long days of summer also occur when the short daylight period of winter is prolonged by use of electric light of low intensity.

To what extent similar changes can be influenced in the normal physiology of growing plants through changes in temperature and food supply must be left for future experiments. Apparently, however, as indicated earlier in this discussion, these factors even though they may be able to operate in some degree are for many plants less important and the reactions to them are less sharply definite than is the case with the effect of the duration of the daily period of light.

PHOTOPERIODISM AND PLANT BREEDING

To attempt to point out the practical significance of a new factor hitherto un-

recognized in biology is as useless as would have been the attempt to predict the wireless telegraph and telephone at the time of the discovery of Hertzian waves. With the proper understanding of this new principle of plant growth, however, it would seem that not only should the work of plant breeders be greatly simplified and clarified but the introduction of crops into new areas might be somewhat less a matter of chance than it is at present. Seed production, especially of highly specialized varieties of plants, should be greatly facilitated. A variety of tobacco, the Maryland Mammoth, is now established agriculturally solely through the utilization of the length of day response. In southern Maryland this type of tobacco grows to an unusually large size, frequently producing more than 100 leaves to a single plant. Because of these characteristics this variety is greatly desired for southern Maryland conditions (plate 8, **A**). However, in Maryland, it usually does not flower at all, or flowering occurs so late that seed will seldom mature. As this variety of tobacco was found to be a short-day plant, (plate 8, **B**) the problem of securing seed is satisfactorily met by growing the seed crop in Southern Florida during the winter, for under that daylight period the Mammoth variety does not continue its vigorous giant growth but flowers and fruits in practically the same manner as other ordinary varieties of tobacco.

LITERATURE LIST

(1) Abbe, Cleveland. 1905. A first report on the relations between climates and crops. U. S. Dept. Agr. Weather Bur. Bul. 36.

(2) Adams, J. 1923. The effect on certain plants of altering the daily period of light. Annals Bot. 37:75–94.

(3) Allard, H. A. 1919. Gigantism in *Nicotiana tabacum* and its alternative inheritance. Amer. Nat. 53:218–233.

(4) Anonymous. 1914. Electric light and sunlight for plants. Jour. Franklin Institute 198:84.

(5) Astruc, A. 1903. Recherches sur l'acidité végétale. Ann. Sci. Nat. Bot., sér. 8, 17:1–108.

(6) Atkins, W. R. G. 1912. The hydrogen ion concentration of plant cells. Sci. Proc. Roy. Dublin Soc., n.s. 16:414–426.

(7) Bailey, L. H. 1891. Some preliminary studies of the influence of the electric arc light upon greenhouse plants. N. Y. Cornell Agr. Exp. Sta. Bul. 30.

(8) ———. 1892. Second report upon electrohorticulture. N. Y. Cornell Agr. Exp. Sta. Bul. 42.

(9) ———. 1893. Greenhouse notes for 1892–93. I. Third report upon electrohorticulture. N. Y. Cornell Agr. Exp. Sta. Bul 55.

(10) Bernard, Noel. 1902. Etudes sur la tuberisation. Rev. Gen. Bot. 14:5–25; 58–71; 101–119; 170–183; 219–234; 269–279.

(11) Bernard, Noel. 1902. Conditions physiques de la tuberisation chez les vegetaux. Compt. Rend. Acad. Sci. (Paris), 135:706–708.

(12) Bonnier, Gaston. 1895. Influence de la lumiere electrique continue sur la forme et la structure des plantes. Rev. Gen. Bot. 7:241–257; 289–306; 332–342; 409–419.

(13) Bouché. 1875. [Ueber das Clandestiniren und Verkummern der Bluthen.] Bot. Ztg. 33:121.

(14) Bovie, W. T. 1915. A direct reading potentiometer for measuring and recording both the actual and the total reaction of solutions. Jour. Med. Research 33 (n.s. 28): 295–322.

(15) Briggs, Lyman J., and Shantz, H. L. 1916. Hourly transpiration rate on clear days as determined by cyclic environmental factors. Jour. Agr. Res. 5:583–649,

(16) ———. 1917. Comparison of the hourly evaporation rate of atmometers and free water surfaces with the transpiration rate of *Medicago sativa*. Jour. Agr. Res. 9:277–292.

(17) Chandler, W. H. 1914. Sap studies with horticultural plants. Mo. Agr. Exp. Sta. Res. Bul. 14.

(18) Clevenger, Clinton B. 1919. Hydrogen-ion concentration of plant juices. Soil Sci. 8: 217–241.

(19) Corbett, L. C. 1899. A study of the effect of incandescent gas light on plant growth. W. Va. Agr. Exp. Sta. Bul. 62.

(20) Coville, Frederick V. 1910. The influence of cold in stimulating the growth of plants. Jour. Agr. Res. 20:151–160.

(21) Flammarion, Camille. 1898(?) Physical and meteorological researches, principally on solar rays, made at the Station of Agricultural Climatology at the Observatory of Juvisy. Exp. Sta. Rec. 10:103–114, 203–213.

(22) Garner, W. W., Allard, H. A., and Foubert, C. L. 1914. Oil content of seeds as affected by the nutrition of the plant. Jour. Agr. Res. 3:227–249.

(23) Garner, W. W., and Allard, H. A. 1920. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Res. 18:553–606.

(24) ———. 1921. Flowering and fruiting of plants as controlled by length of day. U. S. Dept. Agr. Yearbook 1920:377–400.

(25) ———. 1923. Further studies in photoperiodism, the response of the plant to relative length of day and night. Jour. Agr. Res. 23:871–920.

(26) Garner, W. W., Bacon, C. W., and Foubert, C. L. 1914. Research studies on the curing of leaf tobacco. U. S. Dept. Agr. Bul. 79.

(27) Girou De Buzareingues. Ch. 1831. Suite des expériences sur la generation des plantes. Ann. Sci. Nat. 24:138–147.

(28) Haas, A. R. C. 1920. Studies on the reaction of plant juices. Soil Sci. 9:341–368.

(29) Heyne, Benjamin. 1815. On the deoxidation of the leaves of *Cotyledon calycina*; in a letter to A. B. Lambert, Esq., Vice President of the Linnean Society. Trans. Linn. Soc., London 11:213–215.

(30) Hoffman, H. 1882. Thermische Vegetationconstanten; Sonnen- und Schattentemperaturen. Ztschr. österr. Gesell. Met. 17:121–131.

(31) ———. 1886. Phanologische Studien. Met. Ztschr. 3:113–120.

(32) Karling, John S. 1925. A preliminary account of the influence of light and temperature on growth and reproduction in *Chara fragilis*. Bul. Torrey. Bot. Club 51:469–488.

(33) Keeble, Frederick. 1910. Plant-Animals. A Study in Symbiosis. Cambridge Press.

(34) Kinman, C. F., and McClelland, T. B. 1916. Experiments on the supposed deterioration of varieties of vegetables in Porto Rico, with suggestions for seed preservation. Porto Rico Agr. Exp. Sta. Bul. 20.

(35) Klebs, Georg. 1918. Über die Blütenbildung von *Sempervivum*. Flora, N. F. B. 11/12: 128–151.

(36) Klebs, Georg. 1913. Fortpflanzung der Gewächse. 7. Physiologie. Handworterbuch Naturwiss 4:276–296.

(37) Kraus, Gregor. 1884. Ueber die Wassertheilung in der Pflanze. IV. Die Acidat Deszellsaftes. Abhandl. Naturf. Gesell. Halle, 16:141–205.

(38) Laurent, Émile. 1887. Recherches expérimentales sur la formation d'amidon dans les plantes aux depens de solutions organiques. Bul. Soc. Roy. Bot. Belg. 26: 243–270.

(39) Lewis, I. F. 1914. The seasonal life cycle of some red algae at Woods Hole. Plant World 17:31–35.

(40) Liebig, Justus. 1842. Die organische Chemie in ihrer Anwendung auf Agricultur und Physiologie. Aufl. 4. Braunschweig.

(41) Linser, Carl. 1867. Die periodischen Erscheinungen des Pflanzenlebens in ihrem Verhältniss zu den Wärmeerscheinungen. Mém. Acad. Imp. Sci. St. Petersb., s. 7, t. 11, no. 7.

(42) ———. 1869. Untersuchungen über die periodischen Lebenserscheinungen der Pflanzen. Zweite Abhandlung. Mém. Acad. Imp. Sci. St. Petersb., s. 7, t. 13, no. 8.

(43) Livingston, Burton E. 1906. The Relation of Desert Plants to Soil Moisture and to Evaporation. Carnegie Inst. Wash. Pub. 50.

(44) ——— and Brown, William H. 1912. The relation of the daily march of transpiration to variations in the water content of foliage leaves. Bot. Gaz. 53:309–330.

(45) Lodewijks, J. A., Jr. 1911. Erblichkeitsversuche mit Tabak. Ztschr. Induk. Abstam. u. Vererb. 5:139–172; 285–323.

(46) Loeb, Jacques. 1922. The explanation of the colloidal behavior of proteins. Science, n.s. 56:731–741.

(47) Lubimenko, V., and Szboloff, O. 1913. Sur l'adaptation des plantes à la durée de la période claire de la journée. Compt. Rend. Acad. Sci. (Paris) 176:1915–1918.

(48) MacDougal, Daniel Trembly. 1903. The influence of light and darkness upon growth and development. Mem. N. Y. Bot. Gard. 2.

(49) ———. 1920. Hydration and Growth. Carnegie Inst. Wash. Pub. 297.

(50) Magrou, Joseph. 1921. Symbiose et tubérisation. Ann. Sci. Nat. Bot. sér 10, 3: 181–296.

(51) Marcovitch, S. 1923. Plant lice and light exposure. Science, 58:537.

(52) Mayer, Adolf E. 1875. Ueber die Bedeutung der organischen Sauren in den Pflanzen. Landw. Vers. Stat. 18:410–452.

(53) ———. 1878. Ueber die Sauerstoffausscheidung einige Crassulaceen. Landw. Vers. Stat. 21:277–347.

(54) ———. 1884. Kleine Beitrage zur Frage der Sauerstoffausscheidung in den Crassulaceenblattern. Landw. Vers. Stat. 30: 217–226.

(55) McClelland, T. B. 1924. The photoperiodism of *Tephrosia candida*. Jour. Agr. Res. 28:445–460.

(56) McPhee, Hugh. 1924. The influence of environment on sex in hemp, *Cannabis sativa* L. Jour. Agr. Res. 28:1067–1080.

(57) Möbius, M. 1892. Welche Umstände befördern und welche hemmen des Blühen der Pflanzen. Biol. Centbl. 12: 609–624; 673–687.

(58) Molliard, Marin. 1903. Sur l'obtention de bulbes chez l'oignon en cultures aseptiques. Bul. Soc. Bot. France 50:631–633.

(59) ———. 1907. Action morphogénique de quelques substances organiques sur les végétaux superieurs. Rev. Gen. Bot. 19:241–291; 329–349; 357–391.

(60) Moores, Charles A. 1908. The soy bean. A comparison with the cowpea. Tenn. Agr. Exp. Sta. Bul. 82.

(61) Nightingale, G. T. 1922. Light in relation to the growth and chemical composition of some horticultural plants. Proc. Amer. Soc. Hort. Sci. 1922:18–29.

(62) Oakley, R. A., and Westover, H. L. 1921. Effect of the length of day on seedlings of alfalfa varieties and the possibility of utilizing this as a practical means of identification. Jour. Agr. Res. 21:599–608.

(63) Purievich, K. 1893. Die Bildung und Zersetzung der organischen Sauren bei den hoheren Pflanzen. Kiew. Russian original not seen. Abs. in Bot. Centbl. 58: 368–374. 1894.

(64) Rane, F. William. 1894. Electrohorticulture with the incandescent lamp. W. Va. Agr. Exp. Sta. Bul. 37.

(65) Reaumur, R. A. F. de. 1738. Observations du thermometre, faites à Paris pendant l'année mdccxxxv. Comparees avec celles qui ont été faites sous la ligne, à l'Isle de France, à Alger, et en quelques unes de nos Isles de l'Amerique. Hist. Acad. Roy. Sci. (Paris). Mém. Math. & Phys., ann. 1735, p. 545–376.

(66) Reed, Howard S. 1919. Certain relationships between the flowers and fruits of the lemon. Jour. Agr. Res. 17:153–165.

(67) ———. 1921. Growth and sap concentration. Jour. Agr. Res. 21:81–98.

(68) Richards, Herbert M. 1915. Acidity and Gas Interchange in Cacti. Carnegie Inst. Wash. Pub. 209.

(69) Rivera, Vincenzo. 1920. Fattori biologici di rendimento agrario nel mezzogiorno. Riv. di Biol. 2:153–172.

(70) Schaffner, John H. 1923. The influence of relative length of daylight on the reversal of sex in hemp. Ecology 4:323–334.

(71) Schertz, F. M. 1921. A chemical and physiological study of mottling of leaves. Bot. Gaz. 71:81–130.

(72) Schübeler. 1879. The effects of uninterrupted sunlight on plants. (Abstract.) Nature 21:311–312.

(73) Spohr, H. A. 1914. The Photolysis of Plant Acids. Carnegie Inst. Wash. Yearbook 12 (1913): 82–83.

(74) Tincker, M. A. 1924. Effect of length of day on flowering and growth. Nature 114:350–351.

(75) Tournois, J. 1911. Anomalies florales du houblon japonais et chanvre déterminées par des semis hâtifs. Compt. Rend. Acad. Sci. [Paris] 153:1017–1020.

(76) ———. 1912. Influence de la lumiere sur la floraison du houblon japonais et du chanvre. Compt. Rend. Acad. Sci. [Paris], 155:297–300.

(77) Vöchting, Hermann. 1887. Ueber die Bildung der Knollen. Cassel. Bibliotheca Bot. Heft. 4.

(78) ———. 1902. Ueber die Keimung der Kartofferlknollen. Bot. Ztg. 60:87–114.

(79) Vries, Hugo de. 1884. Ueber die periodische Saurebildung der Fettpflanzen. Bot. Ztg. 42:337–344; 353–358.

(80) Wann, Frank B. 1925. Some of the factors involved in the sexual reproduction of *Marchantia polymorpha*. Am. Jour. Bot. 12:307–318.

(81) Wanser, H. M. 1922. Photoperiodism of wheat; a determining factor in acclimatization. Science, 56:313–315.

(82) Warburg, Otto. 1886. Ueber die Bedeutung der organischen Säuren für den Lebensprozess der Pflanzen (Speziell der sog. Fettpflanzen). Unters. Bot. Inst. Tübingen 2:52–150.

(83) Wiesner, J. 1907. Der Lichtgenuss der Pflanzen- ... Leipzig.

A. Violet. Plant at left in flower shows the characteristic behavior under the relatively short days of spring and fall. The plants in the center and at the right were exposed to electric light from sunset till midnight as a supplement to winter daylight. Beginning January 30, however, the daily light period of the center plant was reduced to 7½ hours. In a few days there was partial loss of turgidity in the leaf stems, resulting in the prostrate condition to be noted in the photograph. Subsequently the characteristic blue blossoms of spring appeared in this plant. This prostrate type of development is a characteristic feature in the development of many plants under short-day conditions. Photographed April 17.

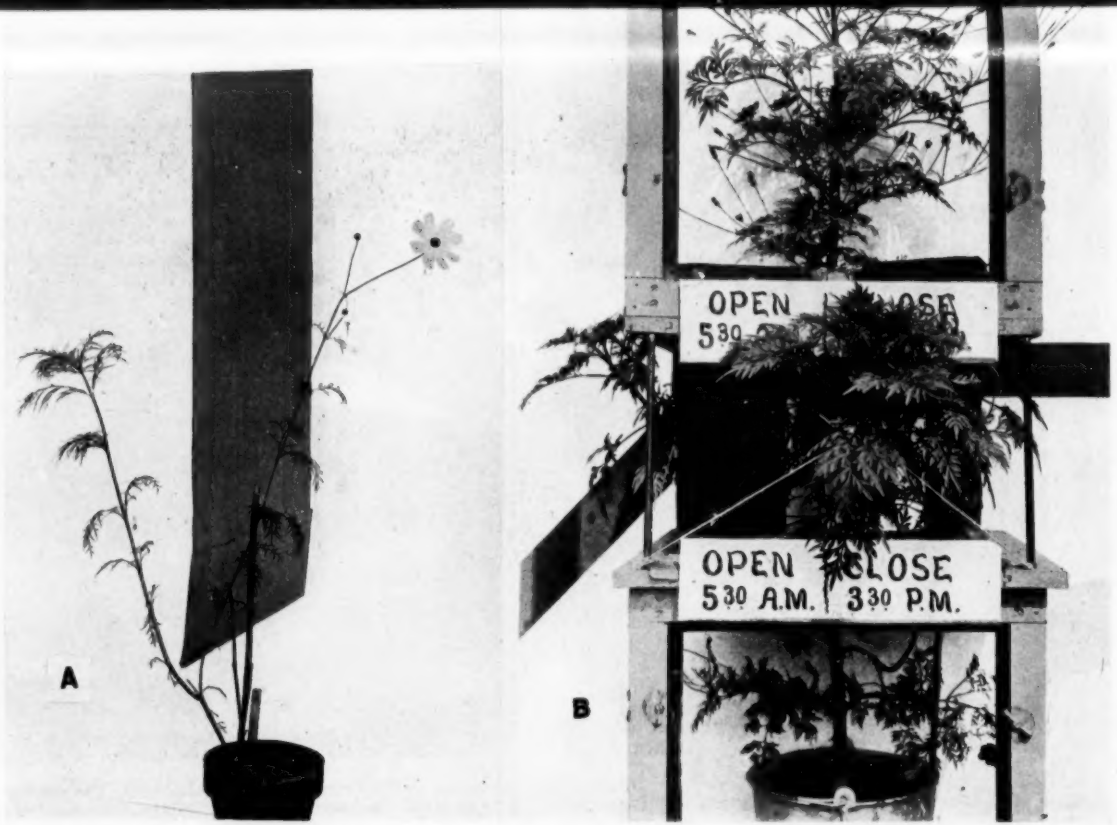
B. Biloxi soybeans. Plants at left exposed to a 12 hour light day. Plants at right exposed to 10 hours of light in two daily periods separated by 4 hours of darkness, thus giving the effect of a 14-hour light day. If the 10 hours of light had been continuous, these plants would have flowered and fruited before those at the left.

A. Cosmos. Planted November 1, plants at right under natural short day length of winter (about 9½ hours) flowered December 22. Under long day exposure (left) plants grew all spring and summer, attaining height of 15 feet.

B. Sunflower. Plants at right were exposed to the full daylight period, supplemented with electric light from sunset till midnight, throughout the test, which began January 27. Plants at left were similarly treated till September 2, when they were given a 10-hour daily light exposure. First blossoms appeared on these plants October 4.

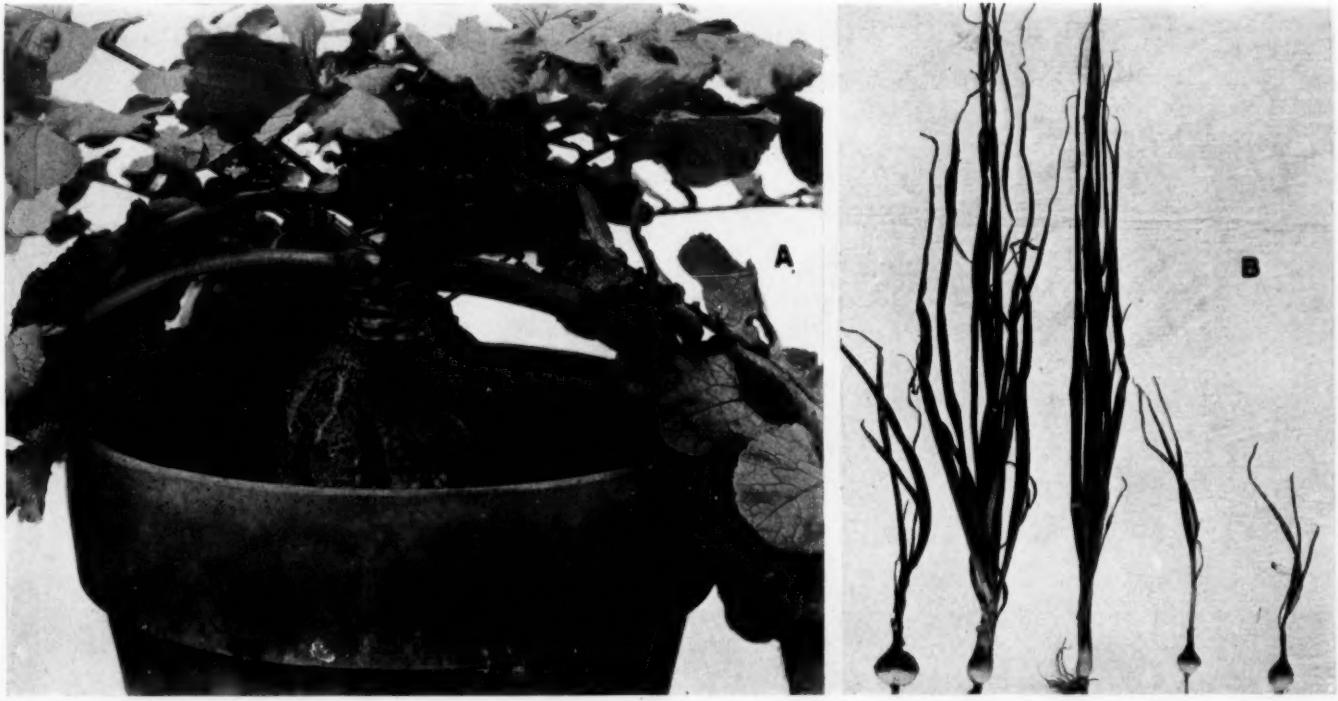
A. Goldenrod. Plant at left received electric illumination from sunset until midnight in addition to the winter days. Plant in center received natural day length, which by March 10 had increased sufficiently to start the growth of flowering stems. Plant at right received only 7½ hours of light throughout the experiment.

B. Lance coreopsis. Beginning November 4 seedling at left exposed to electric light from sunset to midnight in addition to natural day length. Flowering began May 1. Plant at right under natural length of day (about 14½ hours) shows no indications of flowering.



A. Common cosmos. The branch at right flowered promptly in response to the short winter day (about 9½ hours) to which it was exposed. The branch at left of screen remained sterile and continued to grow in response to the added electric illumination from sunset till midnight.

B. Yellow cosmos, the upper and lower portions of which were placed in light-proof chambers on July 11 and thereafter received 10 hours of light daily, while the central portion continued to receive the light of the entire summer day (about 14 hours). Both top and bottom of the plant responded in characteristic manner to the short day and soon flowered, as shown in the photograph. The central portion, on the other hand, remained vegetative in response to the long day.



A. Globe radish, given daily light of 7 hours. About 6 months old.

B. Silverskin onion plants from sets planted May 19, when the day is about 14½ hours long. Plant at extreme left is representative of a series exposed to the full seasonal length of day out of doors. These plants flowered in July, formed bulbs, and passed through the usual summer rest period after the tops had died down. In the series represented by the second plant from the left, which was exposed to a 13-hour day, the growth of tops was much greater while the bulb was delayed in forming and was reduced in size. The plant in center is representative of those exposed to a 10-hour day. In this case there is no summer rest period and no bulb is formed, the tops remaining green indefinitely. The individual at extreme right shows the behavior of plants grown under the natural length of day in the greenhouse, where the temperature was considerably higher than out of doors. The behavior was the same as that of the controls in the open except that the size attained was materially reduced. With the addition of electric illumination from sunset till midnight in the greenhouse only a slight attenuating effect was observed, as shown by the second plant from the right.

A. Seedlings of Baldwin apple. Plants at right were exposed to 10 hours of light daily during the summer months; those on left received the full daylight exposure of summer. It is apparent that growth is more rapid and vigorous with only 10 hours of light daily than with the 14 to 15 hours of daylight of summer in the latitude of Washington. This would indicate a tendency toward an increased rate of growth with decrease in latitude.

B. Seedlings of box-elder. The plants at left were exposed to a 10-hour day while the plants at right were exposed to the full day length of summer. It is obvious that in contrast with the apple this species grows vigorously under long-day conditions.

A. Sumac. These plants were transplanted in April and remained out of doors till September 8, when they were transferred to the greenhouse. They received the full seasonal length of day throughout the test. The leaves were shed at about the usual time, and the plants remained dormant till spring.

B. Sumac, treated in all respects like the plants in **A,** except that when transferred to the greenhouse in September the trees received electric illumination from sunset till midnight. The leaves were retained through the winter.

A. Crop of Maryland Mammoth tobacco growing in southern Maryland where the plants seldom flower or mature seed.

B. Maryland Mammoth tobacco. The plant at the left received only the daylight period of the winter day in the greenhouse; the plant at the right was grown under the same conditions, except that the light period was lengthened by the use of electric light, and the condition of the summer day in Maryland thus stimulated.

RECENT DISCOVERIES IN THE BIOLOGY OF AMEBA

By A. A. SCHAEFFER

Zoölogical Laboratory, University of Kansas

THE ameba, from the moment of its discovery to the present, has stirred the imagination of biologists. Roesel von Rosenhof, that indefatigable amateur naturalist, who first saw and described a large ameba in 1755 (der kleine Proteus) was fascinated by this extraordinary creature which was so different from any living thing he had seen before, for, he says, he "frequently spent two or three hours in observing it." He saw and described the more conspicuous characteristics of the ectoplasm and the endoplasm, the crystals, the manner of movement and, surprisingly enough, he records and figures a case of reproduction by division, and relates how he performed microdissection on an ameba by means of a sharply pointed quill. This must be considered a most excellent contribution to the knowledge of the ameba by a man whose profession was the wholly unrelated one of miniature painting and whose hobby was not protozoa but insects.

The word "ameba" is used as a common noun in this and my previous papers to refer in a general way to any or all of the species of the several dozen genera comprised under the sub-order Amoebaea. A review of recent work on the ameba will perhaps be more illuminating if one recalls something of the historical background for the numerous investigations on this organism within the past few decades, for the ameba and its immediate relatives have figured prominently in the development of some of the most important principles underlying the science of biology. Let us therefore continue for a few moments the historical account of how the ameba has come to be one of the classic organisms for the investigation of the fundamental properties of protoplasm.

After Roesel, nothing of importance was added to the knowledge of the ameba until it came under the eye of the great French naturalist Dujardin in 1841, who, because of his studies of the pseudopods of amebas and foraminifera, made the profound discovery that the gelatinous substance of these organs, the protoplasm, is the physical basis of life. For boldness of conception, far reaching significance and liberating quality, this remarkable concept may well be considered without a parallel in the history of biology. It required the living matter in its pure form, so to speak, as it is observed in amebas and foraminifera to disclose the great fundamental truth that was destined to destroy the mass of mystical and fanciful notions which for a long time had kept the study of living organisms from becoming scientific.

This discovery inevitably began to transform biology from a study of organisms as a collection merely, of curious or interesting objects, to the serious study of the properties of living matter. With the extension of Dujardin's conception by Max Schultze, another great student of the rhizopods, who demonstrated the general identity of plant and animal protoplasm, the ameba, as indeed every other organism, could be studied not merely to

understand that organism, but to explain at the same time the manifestations of protoplasm in any plant or animal whatsoever.

It required, however, the establishment of the principle of evolution to justify completely the method of reasoning from one organism to another with respect to the properties of living matter. The present-day biologist would no more think of questioning the soundness of this form of reasoning than he would question the validity of the multiplication table. Every organism is a "flower in the crannied wall."

With the general acceptance of the principle of evolution the ameba suddenly became the centre of new interest, for, because of its extremely simple structure, it necessarily found itself at the root of the family tree representing the primitive ancestor of man and the animals; and it naturally became of great importance theoretically, therefore, to know more about the structure and especially the physiology and the behavior of this living representative of the most ancient ancestor of man.

The most distinctive property of the ameba is of course its peculiar manner of movement due to protoplasmic streaming, but this property which was first described by Roesel, was later found to be present in greater or less degree in the protoplasm of a great many other organisms, plants as well as animals; and it is now supposed by many zoologists to be essential in the embryological differentiation of the higher animals. The results of investigations on ameboid movement therefore have always been of very wide interest, and this phenomenon is still best studied in the ameba. Many physiologists also hold that the simplest form of muscular contraction, as well as ciliary and flagellar contraction, is some form of ameboid movement and every contribution to the knowledge of ameboid movement is believed also to contribute to an explanation of these forms of contraction. And finally, the discovery of a number of parasitic species of amebas has greatly increased the interest in these organisms from still another point of view. Because of the prominent part the ameba has thus played in the development of the basic conceptions of zoology, it takes its place with the dog and the frog in the great "Zoological Trinity," as outstanding inspirers of basic research.

CYTOPLASMIC DIFFERENTIATION.

In a discussion of the recent advances in the knowledge of the structure of the ameba, it may be well to take up first those parts of its morphology which were discovered first, namely, the ectoplasm and the endoplasm. Here, unfortunately, confusion has arisen as to the meaning of these two words. Thus the word ectoplasm is used to denote, for example; 1, the outer clear layer of an ameba as distinguished from the granular contents; 2, the outer stiffer layer; 3, the clear part of the outer stiffer layer; 4, clear protoplasm wherever it occurs in the ameba. And by implication the endoplasm is, of course, in each case the rest of the protoplasm. Now it goes without saying that it is clearly in the interest of science to use these words with definite meanings, and in this case it seems best to follow the rule in such matters, namely, adopt the original meaning proposed for the words, if practicable. Haeckel originally proposed the words and defined ectoplasm as the stiffer outer layer while endoplasm referred to the internal more fluid layer. These are the meanings adopted in this paper. In addition, the qualifying terms, "clear ectoplasm," "granular ectoplasm," etc., will be found useful, since there is

wide variation in the amount and distribution of granules in both the ectoplasm and the endoplasm of the various species of amebas.

The conversion of endoplasm into ectoplasm at the anterior end of an ameba and the reverse process at the posterior end, producing thus a kind of circulation as the ameba moves along, was first clearly enunciated by Bütschli, but the occurrence of this process in those amebas with a very stiff ectoplasm was questioned by Jennings ('04), who believed the ectoplasm to be something like a permanent sac in which the ameba rolled along, as it were. The evidence for this conclusion consisted largely in the observation of the movement of small particles on the surface of the ameba during locomotion. This quality of the ectoplasm has been investigated recently by Howland ('24), who used a microdissector in her work. Numerous experiments confirmed the generally accepted belief that the ectoplasm in *Thecamoeba* (*Amoeba*) *verrucosa* is very firm, a convincing demonstration consisting in rupturing the ectoplasm and then pulling it off the endoplasm in toto as one might pull an inverted sack off its contents. Howland also showed that ectoplasmic wounds heal rapidly and that large areas of new ectoplasm are made during every act of defecation. It had been observed before that ectoplasm is made and a little later destroyed, whenever a food cup is made for the purpose of eating a particle of food.

The change from ectoplasm to endoplasm and the reverse has also been subjected to experimental analysis, and aside from the effect of change of temperature which has often been noted to change the rate of ameboid movement, presumably by changing the viscosity of the protoplasm, it has been found that by mechanically agitating an ameba the ectoplasm in large part can be transformed into endoplasm even though no locomotion takes place; and by injecting a trace of acid (HCl) into an ameba some endoplasm is transformed into ectoplasm, while the injection of a trace of alkali (NaOH) has the reverse effect (Chambers, '21).

It is of great interest of course to know what factors are concerned in the conversion of ectoplasm into endoplasm and the reverse, since neither the morphology of the ameba nor its characteristic form of movement can be understood unless these factors are known. And it is also recognized that an understanding of the relation between ectoplasm and endoplasm will go far in furnishing an explanation of similar changes in the protoplasm of many other animals and plants, especially in animal eggs at the time of fertilization, mitosis, cleavage, etc. The general problem has therefore been attacked in a number of organisms by experimental methods. The earlier tentative hypothesis in explanation of the difference between ectoplasm and endoplasm was based on a difference in viscosity, which was generally assumed to be brought about normally by some metabolic process, in analogy to changes in viscosity occurring in inert fluids such as pitch, solutions of gelatin and the like. The explanation seemed satisfactory enough while this subject was still in the observational stage; but when the matter began to be tested experimentally by means of micromanipulators in the hands of Kite and Chambers and in various other ways by Hyman, Spek, Heilbrunn and a number of other investigators, it was found that the results of this work did not seem consistent, probably because they did not fall in line with the prevalent simple assumption based on a difference of viscosity. To illustrate: it has been observed that amebas move more slowly

as the temperature is decreased from the optimum towards zero. Many viscous fluids, molasses for example, do the same at the same temperature. One may assume, therefore, as has been done, that the slowing down in the rate of movement is caused merely by a difference in viscosity as a direct result of changes in temperature. But it requires energy to move protoplasm, and this is of course derived from some form of metabolism, that is, chemical change. Now it is also known that such chemical changes as are likely to occur in protoplasm, occur less rapidly or less extensively in lower than in higher temperatures. It is possible therefore that at least a part of the effect of the slowing down of movement with a decrease of temperature is due to a decreasing amount of available energy, and that the temperature effect on viscosity is, to a considerable extent, an indirect effect. There is a considerable body of data bearing on this general subject consisting mostly of isolated observations, but so far it has not been possible to interpret them satisfactorily from a single point of view.

PLASMA MEMBRANE

In addition to the ectoplasm and the endoplasm, which can be distinguished from each other by their physical consistency, there is an extremely thin outer fluid layer on the ameba which can be demonstrated satisfactorily only by its power of carrying small particles adhering to it, toward the anterior end of a moving pseudopod (Schaeffer, '20). Observations and measurements on the activities of this layer indicate that it is the plasma membrane or at least a part of it, and therefore of great physiological interest. It is one of the very few cases where a visible demonstration of this membrane is possible. Since this layer has mistakenly been supposed, in some instances, to include some or all of the stiffer ectoplasm lying immediately underneath, it may be well to state the essential characteristics of this layer as determined by observation (fig. 4).

First, it has been found to exist in a large number of species of amebas of various sizes and shapes. Second, its existence as a structure distinct from the ectoplasm is shown by the fact that particles adhering to it on the outside are seen to move forward over the stationary crystals or other granules imbedded in the ectoplasm, and especially by the rate of movement which in some species (*Thecamoeba sphaeronucleosus*) may be as high as 3.5 times as fast as the ameba itself moves. Third, this layer over the entire free surface of the ameba moves toward the active anterior end at varying rates over different parts of the surface, while the ameba is in locomotion. The substance of the layer must therefore be continually destroyed at the active anterior end of the ameba and be continually recreated at the posterior region of the ameba. Fourth, the layer is extremely thin, being probably less than one micron in thickness. Accurate measurements are difficult to make directly owing to its extreme thinness and to diffraction of the light at the sides of the ameba. The substance of the layer is presumably protoplasmic, and the cause of the movement, if it is a surface tension effect, is due rather to an increase of tension at the anterior end than a decrease in the posterior region, since in those amebas which form ectoplasm to a greater or less extent over the anterior half of the body, the particles move toward the anterior end much less rapidly than in those amebas which form ectoplasm only at the anterior edge.

VARIATIONS IN PSEUDOPODS

One of the most interesting features of the structure of amebas is found in the varied size and shape of the pseudopods,

although we have at present little more than descriptive knowledge of these organs. For convenience of discussion it is well to characterize certain more or less distinctive features of pseudopods by descriptive names. In the first place it is desirable to restrict the name pseudopod to extensions of protoplasm on an ameba greater than a hemisphere. Lesser extensions constantly occur in all amebas and it would be an aid to clearness of description to call these extensions "waves," as some writers, in fact, do. Again, for the sake of clearness, it is not advisable to use the name pseudopod in referring to the whole of a limax ameba; even in amebas it is desirable to indicate whether one is speaking about the whole of one or only a part of the animal.

For a full discussion of the name of the common large ameba (*Chaos diffluens* Müller—*Amoeba proteus* Pallas emend. Leidy emend. Schaeffer) reference may be made to my paper, 1926 Carnegie Institution of Washington Publications, No. 345; and for description of the species reference may be made to my earlier paper ('17) and book ('20). The 1926 paper also contains observations on a number of other species and genera mentioned in the present paper. One cannot observe this ameba in normal movement without noticing that at practically all times some one pseudopod is the main pseudopod through which the ameba flows on. A series of camera lucida sketches of the ameba at intervals of half a minute, for half an hour, illustrates very graphically the presence of a main pseudopod which may preserve its identity for half an hour or more while all other pseudopods within a minute or two after formation are withdrawn. This form of ameboid movement, far from being the general method of locomotion among amebas, is in reality found only in a very few species. A main pseudopod is in fact characteristic of only four other common species: *Metachaos discoides*, *M. annulata*, *M. gratum*, *M. laureata*.

If one sketches, by way of comparison, the form changes of another common large species (*Polychaos dubia*) in a similar way, it is seen that the pseudopods, which at first sight strongly resemble those of the amebas just mentioned, are really formed in quite a different way. No main pseudopod is distinguishable. This ameba, which usually resembles a hand with the fingers spread out, flows forward through two or more pseudopods at the same time, and keeps itself from dividing into several parts by forming web-like extensions continually between the bases of the pseudopods. Although at times one or other of the pseudopods flows more rapidly than the others and thus acquires the character of a main pseudopod, this distinction is only temporary. The torch of leadership is, as it were, continually passing from one to the other among the several leading pseudopods.

Both these types of pseudopods agree, however, in one important characteristic, namely, they increase in size sufficiently to permit the whole ameba to flow through them. That is, they are of *indeterminate* size.

In contrast to these amebas there are a large number of species which form pseudopods that never enlarge beyond a certain size, when they are withdrawn. Such *determinate* pseudopods are characteristic of a common fresh-water ameba of rather small size, *Mayorella bigemma*, and several species of marine amebas belonging to the same genus (fig. 1, *d*). The species *bigemma* is characterized by a triangular or polygonal body while in locomotion, with numerous conical pseudopods scattered over the anterior part of

the body. They are especially conspicuous along the anterior edge. The entire anterior edge of these amebas advances during locomotion although occasionally lobes develop through more rapid flowing of some part of the edge, producing in extreme cases a Y-shaped body. The polygonal shape is, however, quickly resumed again by the withdrawal of one of the limbs of the Y. The ameba thus changed its direction of movement by the unusual method of forming an indeterminate pseudopod. But while this was going on and at all other times of active movement, the advancing edge carried a dozen or more small conical pseudopods which enlarge to a length of 12 or 15 microns and then are withdrawn or they may be continuously projected as small conical points, while the spaces between them advances in the form of a web, for considerable distances. These "false feet" might therefore better be looked upon as "toes" which lead the way in locomotion. There is some property in the protoplasm of this ameba which prevents the advancing wave of protoplasm between the conical pseudopods from catching up with the tips of the pseudopods.

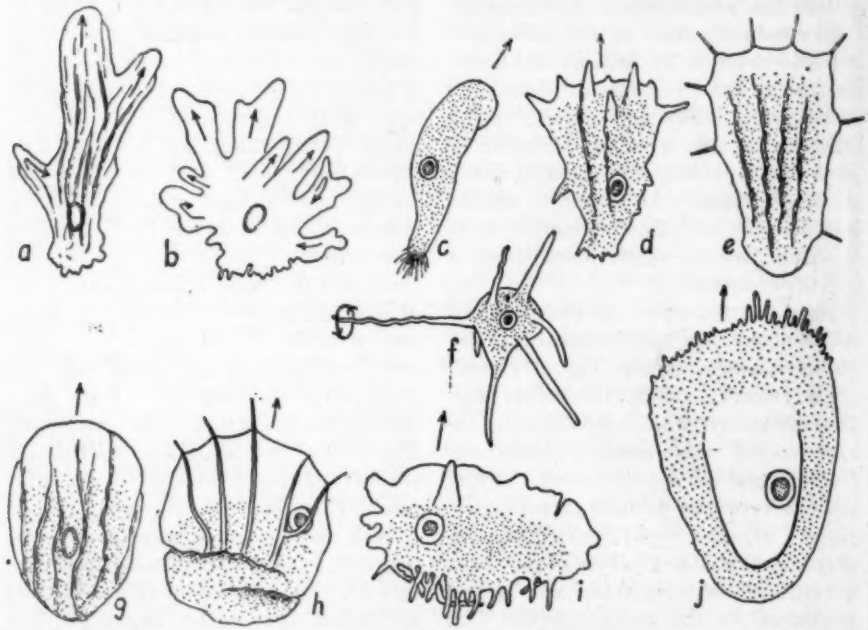


FIG. 1. SKETCHES OF DIFFERENT SPECIES OF AMEBAS TO SHOW VARIOUS KINDS OF PSEUDOPODS AND ECTOPLASMIC RIDGES

a, Chaos diffluens (Amoeba proteus); b, Polychaos dubia; c, Trichamoeba clava; d, Mayorella bigemma; e, Pontifex maximus; f, Astramoeba flagellipodia; g. Thecamoeba verrucosa; h, Hyalodiscus caeruleus; i, Flabellula citata; j, Cochliopodium gulosum. Determinate pseudopods in *d, e, h, j;* indeterminate pseudopods in *a, b.* Note hair-like uroidal projections in *c*, and spiral pseudopod in *f*.

But the most perfect example of determinate pseudopods is found in a large marine ameba which is remarkable from several other points of view. In this ameba (*Pontifex maximus*) in which no

coördinated locomotion has been discovered, small needle-like pseudopods bristle out all over the animal, especially when disturbed (fig. 1, *e*). They resemble somewhat the spines of some cacti. The pseudopods are of clear protoplasm, about 12 microns long by 1 micron thick, and arise from a bulbous base. There is very little variation in size or shape. The first stage in the formation of these pseudopods is the extension of a thin tongue of clear protoplasm, projected into the water from a papilla of clear protoplasm. This tongue is of slightly irregular shape and may wave about slightly in the water. Very soon, however, it reaches the size indicated beyond which it cannot grow and then its protoplasm becomes set and the pseudopod becomes rigid. The protoplasm as it were, has filled the mold; there is nothing to do now but to withdraw. This process is accomplished by a softening of the protoplasm, whereupon the pseudopod becomes crooked and gnarled as it shrinks and finally disappears into the ameba. These determinate pseudopods, each of which represents about 1/100,000 or less of the bulk of the entire ameba, stand in strong contrast to the determinate pseudopods mentioned above which take in the whole ameba, and indicate important structural differences between the protoplasms of the two species of amebas.

The distinction between determinate and indeterminate pseudopods reaches over also into another activity of the ameba, that of eating. It is well known how the common large amebas eat by flowing around the food object, enclosing it in a food cup. One or more pseudopods may take part in the formation of a food cup depending upon the size and location of the food body (Kepner and Taliaferro, '17). But only indeterminate pseudopods can be used in the feeding process. Pseudopods of limited size cannot adapt themselves to the formation of food cups, and amebas which form pseudopods of this character envelop their food by projecting broad waves of protoplasm over and around the food particle, as do also those amebas which form no pseudopods whatsoever (*Thecamoeba verrucosa*) (fig. 1, *g*). Since determinate pseudopods have been found not to take part in either of the two important activities of the ameba, locomotion and feeding, it is difficult to see what function, if any, they perform.

There is another type of small pseudopod which, although it is of determinate character, nevertheless possesses several distinctive qualities which sets it apart from the determinate pseudopods just mentioned. These small pseudopods are the small root-like projections of clear protoplasm which extend backwards from the posterior end of the amebas belonging to the genus *Trichamoeba* (fig. 1, *c*). These projections which may be referred to collectively as the uroid, apparently play a part in locomotion. It has been observed that these projections which are extremely difficult to see in many cases, are fastened to the substratum while the ameba is in locomotion and serve to keep the ameba, which is in clavate shape, from rolling over. But how these uroidal projections release their hold on the substratum and fasten down again, as they obviously must do during locomotion, has not been observed. It has been determined, however, that they may be formed very quickly and that they are probably present in all large clavate amebas with very "fluid" protoplasm. They vary in number from several score or more extremely thin hairlike projections to half a dozen or so larger rootlike and gnarled and, it may be, branched extensions, depending upon the species. These uroidal extensions are of course not to be confused

with the wrinkled remnants of withdrawing pseudopods frequently observed at the posterior region of many large amebas. The character of the uroid is a valuable characteristic in the determination of species.

It is usually assumed that pseudopods move only when or because endoplasm is flowing in or out of them, and this is undoubtedly typical of them. There are exceptions, however. An interesting case is the tentacle-like motility of the pseudopods found in the amebas belonging to the genus *Vexillifera*. These amebas project numerous pseudopods of the determinate type, resembling those of *Mayorella*, but there are usually some at the anterior edge much longer than the rest which wave about in the water more or less actively for 20 seconds or longer before they are retracted. Waving pseudopods are more frequently formed when locomotion has slowed down than when the ameba is moving actively.

Another interesting case of moving pseudopods is found in *Astramoeba flagellipodia*, in which the pseudopods are thrown into loose spirals of three or four turns and kept in motion (fig. 1, *f*). The tip of the pseudopod makes one revolution in about three seconds, the movement being like that of a flagellum. The body of the ameba of course remains stationary during these movements, which makes it clear that the spiral pseudopod is not like a rigid corkscrew being rotated on its long axis, but dynamic, the turns continually forming at the free end and disappearing at the base of the pseudopod. If such a process occurred rapidly the pseudopod would act like a propeller, pulling the ameba through the water. Theoretically, this kind of a pseudopod might perhaps be considered as a stage between the indeterminate pseudopod and a functional flagellum. Static spiral pseudopods are occasionally observed in a number of species of amebas.

All the different kinds of pseudopods mentioned thus far can be projected freely into the water out of contact with a solid body. It remains now to mention the small pseudopods seen on many species of amebas around the periphery of the advancing sheetlike waves of protoplasm, which apparently are not projected freely into the water (fig. 1 *j*). These pseudopods are very difficult to see owing to their great transparency and minute size, being sometimes less than a micron in length. In other particulars, however, they resemble the conical determinate pseudopods described above. These pseudopods are observed most frequently among those amebas that move by means of thin waves of protoplasm, such as *Cochliopodium*, *Gibbodiscus*, *Hyalodiscus*, and some species of *Thecamoeba*.

"HAIRY" AMEBAS

Hairy frogs find their counterpart among the amebas, for there are three species of "hairy" amebas (Wailes; Schaeffer, '25). Two of these species have minute perpendicular hair-like entensions on the outer surface which in one of these species are about one micron long, extremely thin and arranged in a hexagonal pattern with the "hairs" placed one-half micron from each other. Of their nature nothing is known except that they persist after the death of the animal. Another species of ameba belonging to the genus *Pontifex*, has much longer projections. These are ten microns long and one micron thick and cover the outer surface so thickly as to resemble a coat of fur. This resemblance is heightened by the slightly matted appearance of the projections. The color of these projections is a light grayish brown which aids in completely obscuring the underlying protoplasm of the ameba

over considerable areas. These projections also persist after the death of the ameba.

ECTOPLASMIC RIDGES

The ridges which occur normally in the ectoplasm of a number of amebas form an important part in any general discussion of the ectoplasm, but comparatively little attention has been paid to these structures in the past few years. Leidy first gave a good description of the folds in the common large ameba and he also first saw the longitudinal walls of ectoplasm within the general endoplasmic stream. From that time to this, these important structures seem not to have been seen by a single author of a new theory of ameboid movement based upon this ameba, for no mention is made of them!

Longitudinal ectoplasmic ridges are found in a large number of species of amebas. In the family Thecamoebidae, to which the common *Thecamoeba verrucosa* belongs, longitudinal ridges are a family character, and a considerable number of species of the Mayorellidae possess ridges (fig. 1, d, g). Ectoplasmic ridges are not formed in the same way in all cases. In the Thecamoebidae the longitudinal parallel ridges are continually being prolonged at the anterior edge of the ameba and continually destroyed at the posterior end, while the ridges as such retain their identity for many minutes, as long in fact as the ameba retains a more or less straight path. In the Mayorellidae the ridges frequently develop as a buttress extending backwards from a conical pseudopod, which as has been stated above, frequently persists for a comparatively long time. The ridges in the common large ameba are formed in a different way again. In this species the ridges are formed mostly along the whole length at once, by a wave-like outpushing perpendicular to the long axis of the ameba. Occasionally such ridges are prolonged at the anterior end for a short time, but only in rare cases is a ridge found to be as long as the ameba. The part of the ectoplasmic wall bordering on the ridge or lying between two ridges and not rising up with it becomes eventually one of the ectoplasmic walls within the endoplasm described by Leidy.

The width of these ridges is of considerable interest in a study of the general morphology of amebas. How small an ameba may be expected to show sufficient morphological differentiation to enable one to recognize it in the living state? Smaller, apparently, than any ameba now known. The ridges in a new species of marine *Thecamoeba* whose description has not yet been published, are only from 0.5 to 1 micron wide while the ameba itself is only 10 microns wide and long. Another new species closely related to it is 12 microns wide and possesses 3 to 5 characteristic ridges of about the same width. There seems to be no reason therefore why a ridge of the Thecamoeba type should not be found on an ameba as small as 2 or 3 microns, which is the lower size limit for known amebas. In other words there would seem to be no limit imposed upon the existence of recognizable morphological structures in the Thecamoebidae at least, upon which specific distinctions may be based.

This great array of organs which the ectoplasm can construct: the various kinds of pseudopods, uroids, ridges, ectoplasmic walls and "hairs," together with other structures and modifications not here mentioned, in the various species of amebas cannot fail to excite interest in this layer of extraordinary potentialities. The student of the physical properties of protoplasm in so far as form is concerned, can certainly find a rich supply of material in these structures for experimen-

tal work. The ectoplasm possesses its characteristic qualities, however, only because of its position; that is, the endoplasm which may at any moment become ectoplasm possesses the same qualities as the ectoplasm but in a latent form, as it were. But there are distinctive structures of various kinds found in the endoplasm which indicate the existence of still other properties in ameban protoplasm not exhibited by the ectoplasm. We may consider a few of these.

ENDOPLASMIC CRYSTALS

A very common inclusion of the endoplasm are the crystals. They occur in various sizes and shapes depending upon the species. They are found singly almost always, but in *Mayorella bigemma* they are always twinned. So far as known the crystals are enclosed in small watery vacuoles, each in its own vacuole. Their composition is not definitely known although it is believed that one of the phosphates of calcium is the chief, possibly the only constituent.

Curiously enough, the crystals were seen and described (Roesel, 1755) and measured (Ehrenberg, 1838) before the nucleus and the contractile vacuole were seen. After Ehrenberg, however, practically no notice was taken of the crystals until Penard ('02) in his great work on the rhizopods described crystals in several new species of amebas. But Penard was somewhat inclined to doubt whether crystals possessed characteristics stable enough to base specific descriptions thereon. It was found, however, by using the isolation pedigree method with three common species of amebas, that the characters of the crystals are characteristic of the amebas in which they occur, under ordinary conditions (Schaeffer, '17). In later studies a number of other species of amebas were found also to contain crystals whose characteristics possess specific value. In this work a polariscope is very useful because the degree of optical activity of the crystals in at least some species of amebas varies somewhat if abnormal amounts of food are ingested, the crystals under these conditions becoming less clear and sometimes irregular. Most amebas carrying large crystals possess only one kind or size of crystal under average conditions; a few possess a a second or a third kind in small number; while one species, the common *Polychaos dubia*, normally possesses at least four varieties, each of which varies within rather wide limits, the wide degree of variation furnishing here the specific crystal characteristic. In this species the bipyramidal variety predominates, sometimes almost to the exclusion of the other varieties, when food is very scarce and mostly of vegetable nature. In general, however, the crystals vary less under normal or adverse conditions than the pseudopods, the contractile vacuole or even the general shape of the nucleus. Under uniform general conditions the crystals remain uniform.

Very little is known about the origin, significance or fate of the crystals. There are general reasons for believing that they arise as by-products of metabolism and that they are to be regarded as waste products. No instances of the excretion of crystals are recorded, but on the contrary the number of crystals markedly increases in the larger species when division does not take place regularly, so that after a week or two the ameba is little more than a sac of crystals (Schaeffer, '20). This condition is usually fatal. It is probable therefore that crystals are never excreted and that their number is kept down by division of the ameba, reproduction thus being in effect, a form of excretion.

CONTRACTILE VACUOLES

One of the most conspicuous inclusions in the endoplasm of some of the larger fresh water amebas is the contractile vacuole, seen first by Ehrenberg in *Thecamoeba verrucosa*, although nothing of its true nature was recognized at the time. Subsequent observations gradually led to the generalization that fresh water amebas possess contractile vacuoles while salt water forms do not, but recent study of marine amebas has shown that several species of *Thecamoeba* possessing thick ectoplasm contain contractile vacuoles pulsating just like those in the fresh water species. And it was also found that a number of other marine species of amebas when transferred to fresh water formed contractile vacuoles within themselves which pulsated characteristically.

Of the actual structure of the contractile vacuole comparatively little is known. It has been established that the vacuole consists of a drop of fluid which slowly gathers in the endoplasm and is then at intervals emitted to the exterior through a small pore in the ectoplasm. The rate of contraction is much more irregular than that observed in the ciliates, but no detailed study seems to have been made of this function. It has been shown recently that the vacuole is enclosed in an extemporized membrane which preserves its identity when dissected out and placed in the culture medium (Howland).

METAPLASTIC GRAINS

The so-called "excretion spheres," "nutrition spheres" (Hayes), or "metaplasmic grains" are another kind of inclusion within the endoplasm of a large number of species of amebas. These bodies, which range from a fraction of a micron to about 10 microns in diameter, are usually of a bluish or greenish color of varying intensity, depending upon the species, and occur in varying number depending upon the amount of food available. Recently a study has been made by means of stains to learn more about the composition of these bodies, with the result that they seem to be composed mostly of starch (Hayes). It may be mentioned here that in the common large living ameba, these bodies move around slightly in the protoplasm, apparently of their own accord, resembling an exaggerated but slowed down Brownian movement (pedesis), a movement possibly due to differences in surface tension arising out of the deposition or solution of its substance. Similar spheres or grains are found in the pelomyxas, sometimes in large number (*Pelomyxa palustris*, *P. schiedti*), and especially in the parasitic amebas where very large bodies have been described under the name of glycogen. Whatever may be the exact nature of these bodies, there is general concensus of observation that they are reserve food bodies, that they increase in number during periods of abundance of food and decrease in number and size when food is scarce.

Whether the small grains in the peripheral zone of protoplasm of a number of species of *Cochliopodium* have a composition similar to the metaplasmic grains mentioned above is doubtful, because the size of these grains seems to vary very little. These grains are roughly spherical, only a fraction of a micron in diameter and are difficult to see. They are of great interest because they are arranged in one layer only in the thin peripheral zone of otherwise clear protoplasm, at nearly equal distances from each other, in such a way as to lie in rows which radiate in curved lines with their origin in the central mass of protoplasm. The arrangement suggests that of the seeds in the outer

zone of a sunflower head. The peripheral zone of protoplasm is in constant movement while these amebas are in locomotion, nevertheless these grains maintain their arrangement as described. This phenomenon indicates the presence of physical forces in the granules or in the protoplasm which would hardly be suspected if the granules were not limited to one layer by the thinness of the protoplasm of the peripheral zone.

Numerous other kinds of inclusions occurring more or less frequently in the endoplasm such as permanent vacuoles, chromidia, chromatoidal bodies, zoochlorella, symbiotic bacteria, mitochondria, etc., cannot be discussed here with profit owing either to their infrequent occurrence or lack of definite knowledge about them.

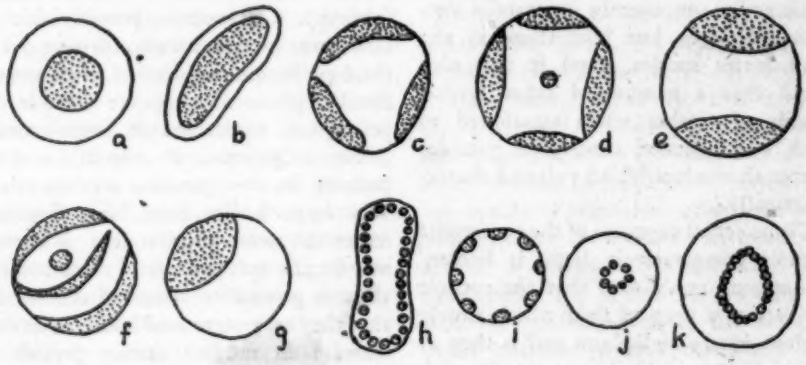


FIG. 2. SKETCHES ILLUSTRATING THE DISTRIBUTION OF PERIPHERAL CHROMATIN IN VARIOUS SPECIES OF AMEBAS, ALL EXCEPT *f* IN OPTICAL SECTION

In single central masses: *a*, *Flabellula citata*; *b*, *Thecamoeba hilla*; in irregular shaped islands on nuclear membrane: *c*, *Rugipes bilzi*; *d*, *Cochliopodium clarum*; *e*, *Thecamoeba munda*; *f*, *Karyamoebina falcata*; *g*, *Endamoeba nana*; in small grains; *h*, *Chaos diffluens*; *i*, *Chaos chaos*; *j*, *Councilmania muris*; *k*, *Mayorella bigemma*.

NUCLEUS

The nucleus is of course the most important of the endoplasmic inclusions because of the essential part it plays in metabolism and reproduction. A nucleus is visible in the living ameba where the nucleus is single or double and not entirely obscured by food bodies. In color it is bluish green or greenish blue, of a somewhat more intense hue than the clear protoplasm, if the chromatin is evenly distributed. If the chromatin is unevenly distributed or exists in discrete grains, the color indicated attaches only to the chromatin. The nuclear membrane is always clear. The nucleus exists in three typical shapes: ovoidal, spherical and discoidal. The best examples of the ovoidal shape are *Polychaos dubia*, whose nucleus is a stout regular ovoid; *Thecamoeba hilla*, whose nucleus has the shape of a slender ovoid that is almost kidney shaped; and *Trichamoeba caerulea* which has about 15 small regular ovoidal nuclei. Discoidal nuclei are found in *Metachaos discoides* and in the common large ameba. In the older and larger individuals of the latter species, the discoid nucleus sometimes becomes folded into an irregular mass. Most of the amebas have a spherical or nearly spherical nucleus, especially the smaller species.

CHROMATIN

With respect to the arrangement of the chromatin in the living "resting" nucleus, four main types occur: 1, in a homogeneous mass in the form of a hollow or an apparently solid sphere or ovoid, with the nuclear membrane immediately outside of it or at some distance from it—*Cochliopodium*, *Hyalodiscus*, *Flabellula*, most *Thecamoeba*, *Endamoeba*—the majority of amebas belong to this group (fig. 2, a, b); 2, in large islands irregularly placed, immediately beneath the nuclear membrane, with or without a clearly distinguishable karyosome in or near the centre (fixing and staining often results in displacing the karyosome into excentric positions)—*Cochliopodium clarum*, *Rugipes bilzi*, *Karyamoebina*, *Metachaos annulata*, *M. gratum*, *Endamoeba nana* (fig. 2, c, d, f, g); 3, in two regularly formed polar caps—*Thecamoeba munda* (fig. 1, e); 4, in a layer of small grains of uniform size at a greater or less distance from the nuclear membrane—*Chaos diffluens* (*Amoeba proteus*), *Chaos chaos*, *Trichamoeba villosa*, *Metachaos discoides*, *Dinamoeba*, *Mayorella bigemma*, *Polychaos dubia* (fig. 1, h, i, j, k). In addition to these masses of chromatin there are found, after fixing and staining, other masses of stainable matter, usually irregularly placed, nearer the centre of the nucleus, and in some species "clouds" or concentric rings of fine dust-like stainable particles occur in the nucleus, whose chromatin constitution has not yet been fully established.

The number of nuclei in the trophic or vegetative stage of amebas varies. The great majority of species have a single nucleus; a considerable number have two nuclei (*Pelomyxa*, *Striolatus*); three species have about 15 nuclei (*Trichamoeba caerulea*, *Pelomyxa belevskii*, *Flamella magnifica*); two species have from 50 to 80, each (*Pelomyxa tertia*, *P. vivipara*); one species, possibly two, have about 125 (*Pontifex*) and three species have from 1000 to 2000 typically formed nuclei (*Chaos chaos*, *Metachaos laureata* and *Pelomyxa palustris*). In one species of ameba (*Astramoeba stella*) the nuclear membrane is single, of oblong shape, but the chromatin is in two masses, indicating probably a long pause in the telophase stage before complete division of the nucleus and of the cytoplasm, since binucleate amebas have never been observed in this species. The condition described is found in about 75 per cent of the amebas of this species. The binucleate condition generally may be explained as differing from the uninucleate only in the long delayed division of the cytoplasm.

CYTOPLASMIC DIVISION

This of course raises the question as to what factors cause cytoplasmic division if it is not the division of the nucleus. Although cytoplasmic division may be induced by a great majority of the nuclei of a multinucleate ameba dividing simultaneously, the considerable variations in number of nuclei observed in the multinucleate amebas indicate that the nuclei frequently divide independently of each other. It is significant too that a large majority of multinucleate amebas frequently become spread out in a thin sheet on the surface of the glass slide and for a longer or a shorter time are incapable of locomotion. While in this condition division frequently occurs in one species as the different marginal regions pull away from each other, and all but complete separation of the cytoplasm occurs in several other species. This phenomenon is very rare among the uninucleate amebas excepting a few of the very small species. There seems to be some connection therefore between the multinucleate condition and the tendency to spread out on the sur-

face, which in some species leads to division. It may be noted here also that the division which Roesel described and illustrated of "der kleine Proteus" is not behavior, which may be regarded as additional evidence that Roesel had before him not one of the species designated *Amoeba proteus* by Leidy, but the much larger and

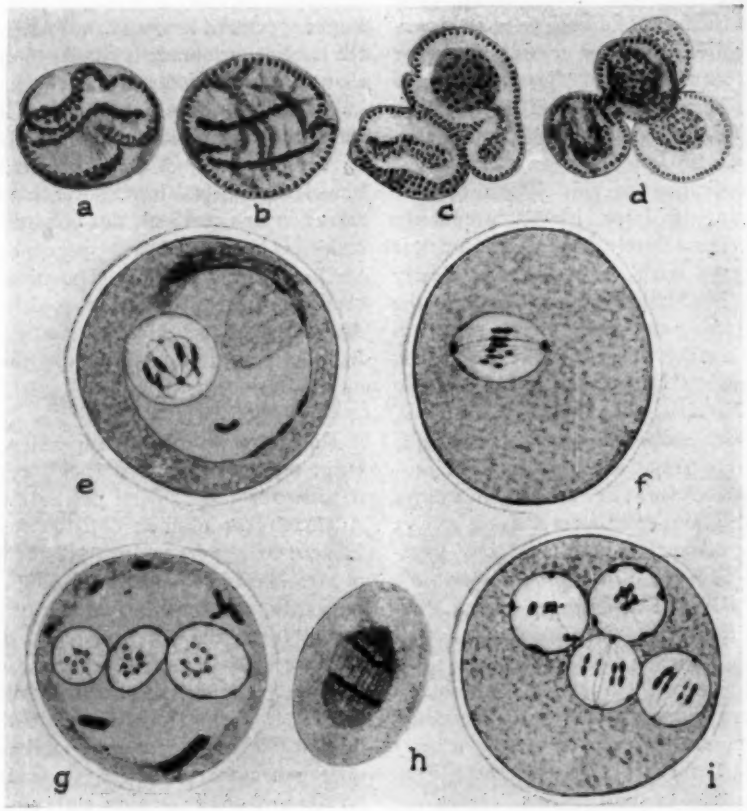


FIG. 3. NUCLEAR DIVISION IN THREE SPECIES OF AMEBAS

a, b, c, d, h, Chaos diffluens. *a*, vegetative divisions; chromatin grains dividing to form two layers; karyosome and membrane also dividing. *b*, complicated vegetative division leading, apparently, to four daughter nuclei. *c*, vegetative division, two daughter nuclei almost separated. *d*, vegetative division; four daughter nuclei almost separated. *e, f, g*, mitosis in cysts of *Councilmania muris*. *e*, nucleus in late prophase. *f*, upper chromosomes in metaphase, lower in early anaphase. *g*, three-nucleate cyst with diffuse karyosome and early stage of intradesmose in right-hand nucleus. *h*, mitosis in *Chaos diffluens* in (probably) a procystic stage, nucleus in anaphase. *i*, *Councilmania decumani*, four-nucleate cyst with two nuclei in metaphase. *a*, *b* (x660); *c*, *d* (x440), after Monica Taylor. *e. f, g. i* (x2500) after Kessel. *h*, after Doflein.

of the type observed in the common large uninucleate ameba but is more or less characteristic of multinucleate ameban rarer multinucleate species which was subsequently rediscovered by H. V. Wilson in 1900.

MITOSIS

One of the most outstanding discoveries on the structure of the nucleus within the past decades has undoubtedly been due to the brilliant investigations of Kofoid and Swezy and their collaborators. They have been able by improved technique to establish a definite number of chromosomes for a number of parasitic amebas, which may very well prove to be the most definite characteristic for identifying these species. The importance of this discovery to parasitology can therefore be readily appreciated. But it is also of great scientific interest in that it furnishes the most definite knowledge we have of mitosis in the amebas, a process which was first discovered to occur in the amebas by Vahlkampf in 1904. Theretofore it was believed that amebas divided amitotically. One needs only to compare the figures of Kofoid and Swezy with those of Vahlkampf, which themselves marked an epoch in this field, to see what tremendous strides have been made in the knowledge of the ameban nucleus in 21 years. We now know that *Endamoeba coli* has 6 chromosomes, *E. dysenteriae* (*histolytica*) probably 6, *Karyamoebina falcata* about 20, *Councilmania muris*, 6 (fig. 3, e, f), *C. decumani*, 4 (fig. 3, i), *C. lafleuri*, 8, (Kofoid, Swezy, Kessel).

This work on the nuclei of these amebas has also cleared up to a large extent other processes in mitosis, such as the origin of the centrosome from the karyosome, the central mass of deeply staining material in the nucleus; the origin and fate of the connecting strand between the centrosomes or intradesmose (Kofoid and Swezy, Kessel) a structure very similar to the centrodesmose in the metazoan cell but apparently more persistent (fig. 3, g); and the splitting of the chromosomes in metaphase. These phenomena are particularly well seen in *Councilmania muris* of rats and mice, in the nuclei of which there is very little peripheral chromatin, almost no clouds of stainable material and in which the karyosome consists of a small number of discrete granules, two of which or the division products of one become the centrosomes. These conditions permit of almost diagrammatic pictures of the mitotic events, so sharply and clearly do these structures stand out. Incidentally it may be noted that the entire mitotic machinery is formed within the nuclear membrane, which must probably be taken to mean that the apparent extra-nuclear origin of the spindle of metazoan cells is a secondary development of the evolution of the mitotic process.

How different is the picture which the common ameba presents! Instead of but six clear cut granules enacting the simple mitotic drama on a perfectly clear stage, we have here several thousand chromatin grains, and a large and complicated karyosome going through a process of division apparently so complicated that it seems to bear only a remote resemblance to the traditional idea of mitosis (fig. 3, *a*, *b*, *c*, *d*). Each of the several thousand chromatin grains, arranged in a single layer underneath the nuclear membrane, divides during fission into two by a plane parallel to the surface of the nucleus, which in this species has a discoidal shape (fig. 3, *a*). The two layers of chromatin grains which result from this division then separate and at the same time the flat disk-shaped karyosome divides into two plates, apparently by a plane perpendicular to the short diameter of the karyosome. The outer layer of chromatin grains then sloughs off while one of the daughter karysomes passes into it, the other karyosome remaining within the inner layer. Thus the two nuclei are formed during division according to the description by Monica Taylor ('24).

Although the main facts involved in this process of division may be definitely stated, the figures show that the process is very complicated and there is no convincing evidence of an actual mitotic process such as Doflein ('18) records (fig. 3, *b*). It must be pointed out however that Doflein fails to show definitely on what species of ameba he worked, and second, he certainly did not work on the vegetative division stages for he says most of his amebas were multinucleate (2 to 8), rounded up and persisted in this shape from one to four days (Schaeffer, '17). It is highly probable therefore that he worked on the shell-less cystlike stage or procysts of the common large ameba, which seem to be a substitute for the shell-bearing cysts of the parasitic species. And Doflein himself doubted whether the mitotic divisions he found represented the only way in which the nucleus of the ameba divided. We therefore still lack sufficient evidence to tell just how our common ameba divides during fission. It would be most extraordinary if after all the nucleus divided amitotically in this stage by means of some process resembling budding as has been suggested. Let us hope that investigations will speedily be initiated to settle this fascinating but tantalizing problem.

CHROMIDIA

The nuclear division processes constitute only a brief stage in the life cycle of the ameba, but we may use these processes as an introduction to the consideration of the other stages in the life cycle. Confining our discussion still to the common large ameba, we may proceed at once to the heart of the matter by asking: Do chromidia give rise to nuclei or do they not? Bott, Popoff, Carter, Ivanic and Monica Taylor ('24) speak affirmatively, and Kofoid ('23), on theoretical grounds, believes the contrary. The origin of nuclei from chromodia has recently been described as follows (Monica Taylor, '24). Some of the several thousand chromatin grains arranged in a layer underneath the nuclear membrane, as described above, are shed into the cytoplasm through rupture of the nuclear membrane. Each grain of chromatin then divides until a cluster of about 8 grains are formed which is then considered as a new nucleus. The new nucleus appropriates some of the ameba's cytoplasm and soon thereafter forms a cyst wall about itself. The ameba becomes filled with hundreds of cysts of this kind (apparently not all of the chromatin grains develop into nuclei) and soon dies and disintegrates, freeing the cysts. The cysts hatch out as amebas and in order to grow to full size require the extraordinary long time of three or four months. Numerous illustrations are given by Taylor. Similar but very indefinite figures are also given by Hausman in a paper on the same subject. This is the affirmative side of the question.

The alternative explanation of the objective events just related is that the small organisms are parasites, as has been pointed out by several investigators. Neither the chromidial nor the parasite hypothesis has however been actually proved. Since the burden of proof in science rests upon him who makes an assertion, no final statement is admissible on this subject unless accompanied by clear proof. The solution of this problem for those who hold the chromidial hypothesis, requires at least the unmistakable identification, as determined by observation, not by deduction, of the chromatin grains with the beginning stage of the small organisms; and second, an isolation pedigree culture of one of these organisms until absolute identity with the parent organism as to details of structure is es-

tablished. It would be more practical perhaps to settle the second point first. Those who hold to the parasite hypothesis must be able to furnish proof by reinfecting other amebas with the supposed parasite. The figures thus far published on these putative young amebas do not convincingly resemble the adult "mother" ameba from which they came. It is only fair to say that Monica Taylor is one of those who recognizes this and is at work in an earnest endeavor to solve this vexatious question.

If we adopt then the critical scientific attitude we find no proof that the life cycle of the common large ameba includes more than the irreducible minimum, namely, reproduction by fission. That is all. Fission may be mitotic or amitotic; we do not know definitely because the various authors did not use pure cultures and most of them did not make it clear which one of three to five possible species were employed. The same statement applies to the accounts of sporulation and encystment.

FLAGELLATE STAGES

Other species of amebas however have more complicated life cycles, as is well known. Thus in the parasitic species reproduction occurs in the encysted stage resulting frequently in 16 small amebas and in other species living in the soil or in cultures of feces outside of the body, a flagellate stage may often be observed. The most interesting case of the latter kind has to do with *Tetramitus*, a flagellate from the feces of the rat, which was described by Perty in 1852. This flagellate of rather complicated structure was found recently to have an ameboid stage in its life cycle. An ameba of typical form hatches out of a cyst. It feeds and divides to form other amebas for a number of generations. Some of these encyst. Others become transformed directly into the traditional flagellate *Tetramitus*. In this stage growth occurs followed by repeated divisions. Sooner or later the flagellates transform directly into amebas which after a time encyst, completing what is known of the cycle. This extremely interesting change from an ameba into a flagellate of rather complicated structure and back again is one of many indications that the amebas and the flagellates are very closely related.

GENETICS

In the field of genetics regrettably little has been done with amebas. Isolation pedigree cultures have been employed to test the specific characters of several amebas and, by means of simple statistical methods, the existence of five races has been indicated in *Endamoeba histolytica* (*dysenteriae*) and four in *E. coli* (Dobell and Jepps). By means of isolation pedigree cultures it has also been found that there are rhythms in the rate of fission in *Mayorella* (*Amoeba*) *bigemma* comparable to those found in the infusoria, but whether they are of the same nature fundamentally has not been determined. The rate of fission in five species pedigreed singly is extremely irregular varying from two to three divisions in 24 hours to one division in 8 to 12 days, at room temperature. The cause of this irregularity is not known (Schaeffer, Botsford).

REGENERATION

The question of regeneration in ameba has received a great deal of attention in the past. After it was established however that the enucleated part of a bisected ameba died after 10 or more days while the nucleated part lived, little was done in this field until very recently. A quarter to a third was cut off an ameba almost daily to see what the effect would be on sub-

sequent reproduction. After a series of such excisions were made, extending over a month, during which time the ameba did not divide, further excisions were discontinued whereupon the ameba grew in size and divided normally. Since it had been shown before that the common large ameba usually dies if it does not divide in 9 days, it seems that excisions are in some way a substitute for divisions (Hartman, Phelps). The interesting observation was also made that of two daughter amebas from the same mother, the one bisected as above indicated and the other allowed to divide normally, the normal one produced five times as much protoplasm as the bisected one. It was found too, that the "minimal reorganization mass," the smallest piece capable of regeneration, was at least as small as 1/80 of the ameba, being therefore the smallest regenerating mass yet recorded among the protozoa (Phelps).

AMOEBOID MOVEMENT

In the foregoing paragraphs are described some of the more recent published researches bearing more or less directly upon the morphology of the amebas, but including also a number of physiological discoveries arising out of these researches. It now remains to consider the results of some investigations on amebas, whose primary object was physiological. For the purpose of general discussion these researches may be divided into two groups: 1, those dealing with ameboid movement and 2, those dealing more particularly with the reactions of amebas toward various stimuli.

Speaking first of ameboid movement, it may be pointed out that the universal interest which this phenomenon excites among biologists is attested by the large number of theories of ameboid movement which have been published. But although these theories reflected interest in the subject, it seems that they did not add to an understanding of it. Just as the 260 or more theories of sex quietly passed into oblivion when the work of Mendel became known, so the theories of ameboid movement are being forgotten as the results of observation and experiment on the movement of amebas are accumulating. We do not therefore think it profitable to discuss any of these theories here, but shall instead devote the available space to objective data which have been rapidly accumulating in the past few years.

The activities of the surface layer of the ameba, that is, the plasma membrane have already been described. Judging from the observations on its behavior, this layer is the surface tension layer and the question arises here whether it is a necessary factor in ameboid movement. Since it does not move in the same way in the pseudopods of *Difflugia* or of foraminifera and is of course absent as a moving layer in ciliates and those plant cells where streaming protoplasm occurs, its movement as seen in the amebas probably does not constitute an essential factor in ameboid movement. But its movements nevertheless indicate that the surface tension is greater at the anterior ends of active pseudopods than over the rest of the ameba, and this fact if correctly interpreted, may prove to be a useful guide in further experimental analysis.

The discovery that amebas move in wavy or sinusoidal paths when comparatively free from external stimulation in clear culture fluid, is of considerable interest because of the probability that this path is a projection of the well known helical spiral paths of ciliates and flagellates on a plane surface, the ameba being of course, restricted in its movements to two dimensions of space (fig. 4). This probability is strengthened by the fact that the flagellate

stages of those amebas which go through these stages move in helical spirals. It has been found also that a great many other organisms, such as the larvae of many species of aquatic invertebrates and all motile spermatozoa move in similar paths. In fact all animals including man and all motile plants move in spirals of some form when the orienting senses are not functioning (Schaeffer, '20). Very little of the nature of this fundamental mechanism affecting movement has been learned excepting that in the ciliates, where an extensive investigation covering 165 species showed, (1) high correlation between the direction of the spiral, whether right handed or left handed, and generic characteristics, that is, the species of most of the genera containing two or more species swim, all either right handed or left handed; (2) the investigation showed no correlation whatever between any visible morphological structures and direction of turning (Bullington). Because of its universal occurrence and the absence of correlation to visible structure, this spiralizing tendency is difficult to investigate experimentally by analytical methods. It appears to be controlled by the spatial aspect of some feature of the locomotive machinery, but with such an enormous variety of locomotive apparatus as is comprised under all the motile animals and plants, it is quite evident that one must go behind visible morphology to stereochemical aspects of the protoplasm, for a cause, at least of the difference between left hand and right hand turning.

Ameboid movement as generally thought of is restricted to those form changes of protoplasm which are due to the streaming of the protoplasm, and as thus conceived the heart of the problem is to discover what causes protoplasmic streaming. All other phenomena associated more or less frequently with streaming, such as the formation of ectoplasm and the conversion of it into endoplasm, are accessory and nonessential, for in some organisms where streaming occurs there is no regular formation of ectoplasm, e.g., plant cells and foraminifera. For practical purposes, however one must confine one's experimental work to one or at most a few forms, and for this reason it becomes necessary and often very desirable to study the accessory phenomena, because these phenomena often are a measure of the streaming activity and serve

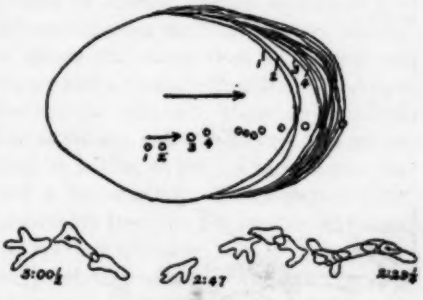


FIG. 4. Above, *Thecamoeba sphaeronucleolus*

Showing movement of the plasma membrane as indicated by a small particle attached to it. Twelve successive outlines of anterior end of ameba are shown, corresponding with 12 successive positions of the particle. The plasma membrane moved over 3.5 times as fast as the ameba. Below, waves in a 30-minute section of path of *Metachaos discoides*.

therefore as a guide in its experimental analysis. But it is nevertheless true that the great interest displayed in ameboid movement is due to its very widespread occurrence in animals and plants, and that the only constituent phenomenon that one can see which is common to all cases of ameboid movement wherever it is found, is streaming.

A number of researches have been recorded in the past few years which, it may be presumed, were directed upon this central problem of ameboid movement. Of these, the investigations of Pantin seem

to have been particularly well planned for they have yielded clear cut and important information on this subject. The work was done on marine amebas which, because of the medium in which they live, offer certain decided advantages over fresh-water species for certain pruposes. One of the very interesting conclusions from this work is that there is a rise in hydrogen ion concentration in an active pseudopod. This was determined by using neutral red as an intra vitam stain.

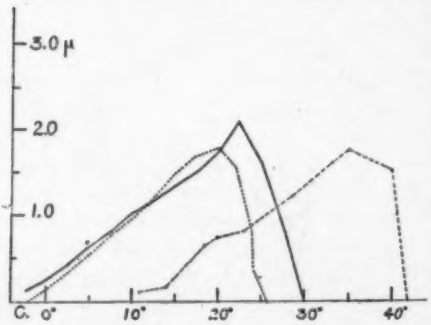


FIG. 5. CURVES ILLUSTRATING THE EFFECT OF TEMPERATURE ON VELOCITY OF THREE SPECIES OF AMEBA

Solid line, "Type A" (*Trichamoeba gumia?*) ameba, after Pantin; dotted line, "type B" ameba, after Pantin; broken line, *Cochliopodium sp.* (velocity multiplied by 1.6. Schaeffer, '24). Note the very suggestive general identity of the high optimum curve of *Cochliopodium* with the other two curves.

It could not be ascertained with certainty, however, whether only the small granules or the fluid cytoplasm, or both, became more strongly acid. By immersing the amebas in various acid solutions it was found that hydrochloric, acetic, butyric, lactic, sulphuric and oxalic acids inhibited ameboid movement, each at about the same rate as shown by the plotted curves. But citric and tartaric were much less effective in inhibiting movement. In these solutions the paralysis point ranged from pH 5 to pH 6, while in the first series of acids the paralysis point was about pH 7. The inhibiting effect seems to be due to the pH concentration, but the protective effect of the citrate and the tartrate remains unexplained.

The effect of temperature on velocity of movement on the same two species of ameba showed the optimum temperature for one species to be from 22°C. to 25°C., and for the other species 20°C. (Pantin) (fig. 5). These optima, it may be remarked, were probably fixed by the temperature of the medium in which they grew, for the optimum temperature as measured by the rate of movement of an ameba living in shallow tidal pools at Tortugas, Florida, in which the temperature rises to 40°C. daily in the sun, is 35°C. or slightly above. The optimum for this ameba is in fact 50 above the death point of the amebas Pantin employed (Schaeffer) (fig. 5). When the rate of movement in various temperatures is plotted against the temperature, the resulting curve to the optimum is almost a linear function of the centigrade temperature and is closely similar to the temperature-velocity curves of many other biological processes, particularly protoplasmic streaming in plant cells and the leucocytes of human blood (Pantin, Schaeffer, Kanitz, McCutcheon).

After the optimum temperature is reached in these amebas, the curve drops rapidly to the base line, an effect which may be due to the destruction of some substance concerned in locomotion. In fact this apparently destructive effect is already present to a slight degree before the optimum is reached, because if the velocity of an ameba is measured at 10°C., for example, and then at 20°C., and then again at 10°C., the velocity is less on reëntering 10°C. Nevertheless within a fraction of an hour the ameba regenerates the substance destroyed and recovers its original velocity.

On the other hand observation indicates that several, perhaps all species of amebas can be acclimated to higher optimum temperatures, which would mean that substances are gradually built up in much the same way that the substance mentioned above was regenerated, but at the same time containing within them the possibility of a destructive effect due to the temperatures near the optimum. Herein are opportunities for whole series of important quantitative experiments on the nature of ameboid movement which would also undoubtedly shed light on other biological processes.

The treatment of amebas with solutions of various chemicals in order to learn something of the nature of ameboid movement, has been carried on by a number of observers in the past few decades. Similar experiments have also been made upon leucocytes of man and other animals for the same purpose. Studies of this sort upon the common large ameba have shown that all chemical solutions thus far accurately tested have had the effect of slowing down the rate of movement, if indeed any effect was detectible at all. This is an extraordinary result, but it is of course only a "progress report," and the final statement on the subject may be different. In recent work (Edwards, Forgrave) with the solutions of the chlorides of sodium, lithium, potassium, etc., it was found that the rate of locomotion of ameba when immersed in them is slowed down in a degree depending upon the cation, the concentration of the solution and the time of immersion. The rate of movement was greatest in solutions of sodium chloride. Acid solutions cause the surface to become sticky in dilutions up to N/20,000. Bases (sodium and potassium hydroxide) on the other hand seem to liquefy the surface protoplasm and make it susceptible to rupture.

One of the interesting results of this work with chemicals is the action of weak (N/300) solutions of the chlorides of sodium, calcium, lithium, potassium, in causing the formation of food cups in ameba. This is the only case known in ameba where a completely generalized stimulus sets into operation what seems to be a comparatively highly specialized "racial habit," which ordinarily is initiated only by localized stimuli (Edwards).

It is interesting to note here that various species of marine amebas representing 3 different familes move much more rapidly in dilute sea water than in normal sea water, and a similar effect was noted in a few marine ciliates. These amebas live only in the sea, so far as known, and one of them is unable to live in fresh water for even a few minutes. These amebas have presumably lived for a long time in the sea and yet they have been unable to overcome the inhibiting effect of the salts in the sea water. This indicates the presence of an unalterable relationship between these salts and an essential reaction involved in ameboid movement and probably also in ciliary contraction (Schaeffer, '24).

BEHAVIOR

In proportion to the total amount of research on amebas, comparatively little has been done in the field of behavior within the past few years. One series of experiments directed upon the ability of the ameba (species not mentioned) to modify its responses has shown that this is possible although the conclusion is somewhat indefinite and the response is of a negative nature, not a positive one. Most of the amebas used in the experiment were negative to large areas of very intense light. It was found that there is a decrease in the number of attempts to continue in the original direction (into the light) as the number of trials increases.

The ameba "learns" to avoid the light (Mast and Pusch).

Another series of experiments on the effect of light on ameba with reference especially to the latent period, reaction time, etc., has shown that in general the ameba reacts substantially like other organisms. The ameba responds to a sudden increase of illumination after a few seconds by a cessation of movement, and this intervening period of time is here known as the reaction time. It is found that the reaction time varies inversely with the intensity of the light. Only a momentary flash of light is needed to inhibit streaming some seconds later. The length of time the light shines is therefore the stimulation period, and the time between the end of the flash and the inhibition of movement is the latent period. The latent period, like the reaction time, varies with the intensity of the light, being short in strong light and lengthening with decreased intensity of light. It was found also that from one to two minutes must elapse after sudden illumination before a second response could be obtained. Mechanical shocks produce effects substantially like the stimuli from light, from which the conclusion is drawn that the effect of light is not necessarily due to the presence of a photo-chemical substance. Perhaps the most unlooked-for result of this work is the absence of any temperature effect on the reaction time. It is however not impossible that the temperature effect may be small and may have remained hidden under the individual variations, which were not inconsiderable (Folger).

In the first of several proposed papers Schwitalla has recorded a large number of detailed observations on the effect of temperature on the movement of *Metachaos discoides* and on the common large ameba as measured by the movement of the posterior end. He records the occurrence of irregular rhythms in the velocity, the cause of which however was not detected. The rhythms of fast and slow movement changed somewhat as the temperature changed, that is, as the temperature increased from 15°C. to 25°C. the fast rhythms became fewer or shorter or both, as compared with the slow rhythms.

Beers records a very interesting case of the common large ameba feeding on the large ciliate *Frontonia leucas*, by pinching it into two, as occasionally happens also with paramecium and blue stentors. Why the ameba should not ingest the whole frontonia or paramecium is hard to see, for much larger food objects are frequently eaten. This raises the question whether the ameba actually pinches the ciliate into two as has so often been assumed. It is more probable that the ciliates constrict themselves when the food cup of the ameba presses lightly against their cilia, as they are often seen to do when threading their way through debris. The body of the ciliate at the point of constriction under these conditions is always smaller than the hole or slit in the debris, so that the animal can readily rotate on its long axis. A time element is of course involved, and the ameba closes in upon the prey slowly enough for such a constricting process by the ciliate to occur. It cannot be accepted without actual proof, therefore, that the ameba actually expends the necessary energy required to cut the ciliate into two instantly. These observations have often been assumed to be particularly fatal to the simple surface tension theory of ameboid movement, but the critical attitude suggested above need occasion no fears as to its possible resurrection, for this theory was already sufficiently dead.

LITERATURE LIST

(Starred references contain extensive bibliographies)

Bebb, C. D. 1924. Observations on amoeba feeding on the ciliate *Frontonia*. Brit. Jour. Exp. Biol., Vol. 1, pp. 335–341. 2 figs.

*Boeck, W. C., and C. W. Stiles. 1923. Studies on various intestinal parasites (especially amoebae) of man. U. S. Pub. Health Bull. No. 133, pp. xxvi + 202. 80 figs.

Botsford, E. F. 1922. Rhythms in the rate of reproduction of *Amoeba bigemma*. Proc. Soc. Exp. Biol. Med., Vol. 19, p. 397.

*Bullington, W. E. 1925. A study of spiral movement in the ciliate infusoria. Arch. f. Protistenk., Vol. 50, pp. 219–274.

Bunting, M. 1922. A preliminary note on *Tetramitus*, a stage in the life cycle of a coprozoic amoeba. Proc. Nat. Acad. Sci., Vol. 8, pp. 294–300.

Carter, L. A. 1919. Some observations on *Amoeba proteus*. Proc. Royal Phys. Soc. Edinburgh, Vol. 20, pp. 193–211. 1 fig.

*Cash, J., and J. Hopkinson. 1905. The British Fresh-Water Rhizopoda and Helioza. Vol. 1, Ray Society. 150 pp., 16 pls.

Chambers, R. 1920. Dissection studies of ameba. Proc. Soc. Exp. Biol. Med., Vol. 18, p. 66.

Chambers, R. 1921. The effect of experimentally induced changes in the consistency on protoplasmic movement. Proc. Soc. Exp. Biol. Med., Vol. 19, p. 87.

Crozier, W. J., and H. Federighi. 1924. Critical thermal increment for the movement of oscillatoria. Jour. Gen. Physiol., Vol. 7, pp. 137–150. 6 figs.

De Haan, J. 1922. Mobilite amiboide et phagocytose. Arch. Neerlandaises de Physiol., Vol. 6, p. 388.

Dobell, C., and M. W. Jepps. 1918. A study of the diverse races of *Entamoeba histolytica* distinguishable from one another by the dimensions of their cysts. Parasitol., Vol. 10, pp. 320–351. 7 figs. 1 pl.

*Dobell, C. 1919. The amoebae living in man. vi + 155 pages. London.

Doflein, F. 1918. Die vegetative Fortpflantzung von *Amoeba proteus* Pallas. Zool. Anz., Vol. 59, pp. 257–268. 12 figs.

Edwards, J. G., and H. S. Forgrave, Jr. 1923. The rate of locomotion of ameba in alkali chlorides. Johns Hopkins Hosp. Bull., Vol. 34, pp. 387–389.

Edwards, J. G. 1924. The action of certain reagents on amoeboid movement. Brit. Jour. Exp. Biol., Vol. 1, pp. 571–595. 1 pl.

Edwards, J. G. 1925. Formation of food-cups in amoeba induced by chemicals. Biol. Bull., Vol. 48, pp. 236–239. 4 figs.

Folger, H. T. 1925. A quantitative study of reactions to light in amoeba. Jour. Exp. Zool., Vol. 41, pp. 261–291. 3 figs.

Fürth, O. 1922. Zur Theorie der Amöboiden Bewegung. Arch. Neerlandaises de Physiol., Vol. 7, p. 39.

Galtsoff, P. S. 1923. The amoeboid movement of dissociated sponge cells. Biol. Bull., Vol. 45, pp. 153–161. 1 fig.

Gerisenko, H. 1922. Untersuchungen zum Plasmabau der Amöben, im Hinblick auf die Wabentheorie. Arch. f. Entw.-Mech., Vol. 51, pp. 150–250.

Goodrich, H. B. 1924. Cell behavior in tissue cultures. Biol. Bull., Vol. 46, pp. 252–262. 9 figs.

Gray, J. 1922. The mechanism of ciliary movement. Proc. Royal Soc., B., Vol. 93, p. 122.

Gray, J. 1923. The mechanism of ciliary movement. Proc. Royal Soc., B., Vol. 95, p. 6.

Haberlandt, L. 1919. Ueber Amöboide Bewegung. Zeitschr. f. Biol., Vol. 69, pp. 409–436.

Hartmann, M. 1924. Der Ersatz der Fortpflanzung von Amöben durch fortgesetzten Regenerationen. Arch. f. Protistenk., Vol. 49, pp. 447–464.

Hausman, L. A. 1920. A contribution to the life history of *Amoeba proteus* Leidy. Biol. Bull., Vol. 38, pp. 340–352. 2 pls.

Hayes, Carmela. 1924. Nutritive spheres in amoeba. Quart. Jour. Micros. Sci., Vol. 69, pp. 144–149.

Hegner, R. W. 1920. The relations between nuclear number, chromatin mass, cytoplasmic mass and shell characteristics in four species of the genus *Arcella*. Jour. Exp. Zool., Vol. 30, pp. 1–95.

Heilbrunn, L. V. 1921. Protoplasmic viscosity changes during mitosis. Jour. Exp. Zool., Vol. 34, pp. 417–447.

Hogue, M. J. 1922. A comparison of an amoeba, *Vahlkampfia patuxent* with tissue culture cells. Jour. Exp. Zool., Vol. 35, pp. 1–11.

Hogue, M. J. 1923. Contractile vacuoles in amoebae-factors influencing their formation and rate of contraction. Jour. Elisha Mitchell Sci. Soc., Vol. 39, pp. 6.

Howland, R. 1924. Dissection of the pellicle of *Amoeba verrucosa*. Jour. Exp. Zool., Vol. 40, pp. 263–270. 15 figs.

HOWLAND, R. 1924. Experiments on the contractile vacuole of *Amoeba verrucosa* and *Paramecium caudatum*. Jour. Exp. Zool., Vol. 40, pp. 251–262. 7 figs.

HYMAN, L. H. 1917. Metabolic gradients in amoeba and their relation to the mechanism of amoeboid movement. Jour. Exp. Zool., Vol. 24, pp. 55–99. 14 figs.

IVANIC, M. 1924. Zur Kenntniss der Fortpflanzungserscheinungen einiger Süsswasseramöben. Arch. f. Protistenk., Vol. 50, pp. 113–134. 24 figs.

*JENNINGS, H. S. 1910. Life and death, heredity and evolution in unicellular organisms. 233 pages. 53 figs.

KEPNER, W. A., AND W. H. TALIAFERRO. 1917. Food-reactions of *Pelomyxa carolinensis* Wilson. Jour. Exp. Zool., Vol. 24, pp. 381–394.

KESSEL, J. F. 1924. The distinguishing characteristics of the parasitic amoebae of culture rats and mice. Univ. Cal. Pub. Zool., Vol. 20, pp. 489–544. 3 figs., 6 pls.

KOFOID, C. A. 1923. The life cycle of the protozoa. Science, N. S., Vol. 57, pp. 397–408.

KOFOID, C. A., AND O. SWEZY. 1924. *Karyamoeba falcata*, a new amoeba from the human intestinal tract. Univ. Cal. Pub. Zool., Vol. 26, pp. 221–242. 2 figs., 2 pls.

LAPAGE, G. 1922. Cannabalism in *Amoeba vespertilio*. Quart. Jour. Micros. Sci., Vol. 66, pp. 669–710. 3 figs., 2 pls.

LOEB, L. 1921. Amoeboid movement, tissue formation and consistency of protoplasm. Amer. Jour. Physiol., Vol. 56, pp. 140–167.

MAST, S. O. 1923. Mechanics of locomotion in ameba. Proc. Nat. Acad. Sci., Vol. 9, pp. 258–261.

MAST, S. O., AND L. C. PUSCH. 1924. Modification of response in amoeba. Biol. Bull., Vol. 46, pp. 55–59. 1 fig.

MCCUTCHEON, M. 1923. Studies on the locomotion of leucocytes, II. Amer. Jour. Physiol., Vol. 66, p. 185.

*NÖLLER, W. 1922. Die wichtigste parasitischen Protozoen des Menschen und der Tiere. I, die parasitischen Rhizopoden. 272 pp. Berlin.

*PANTIN, C. F. A. 1923. On the physiology of ameboid movement. Jour. Marine Biol. Ass. United Kingdom, Vol. 13, pp. 24–69. 10 figs.

PANTIN, C. F. A. 1924. On the physiology of ameboid movement. II. Brit. Jour. Exp. Biol., Vol. 1, pp. 519–538. 4 figs.

PHELPS, L. A. 1923. Experimental analysis of factors concerned in division in ameba. Anat. Record, Vol. 31, p. 312.

REYNOLDS, B. D. 1924. Interactions of protoplasmic masses in relation to the study of heredity and environment in *Arcella polypora*. Biol. Bull., Vol. 46, pp. 106–140.

*SCHAEFFER, A. A. 1920. Ameboid movement. vii + 156 pages. Princeton press.

*SCHAEFFER, A. A. 1926. The taxonomy of the amebas with descriptions of thirty-nine new marine and fresh-water species. Publ. Carnegie Institution of Wash., No. 345 (contains full bibliography of previous papers by this author).

SCHWITALLA, A. M. 1924. The influence of temperature in the rate of locomotion in amoeba. Jour. Exp. Zool., Vol. 39, pp. 465–513. 8 figs.

SWEZY, O. 1922. Mitosis in the encysted stages of *Endamoeba coli*. Univ. Cal. Pub. Zool., Vol. 20, pp. 313–322. 2 pls.

TALIAFERRO, W. H., AND F. O. HOLMES. 1924. *Endamoeba barreti* n. sp. from the turtle, *Chelydra serpentina*. Amer. Jour. Hygiene, Vol. 4, pp. 155–168. 1 pl.

TAYLOR, MONICA. 1918. Note on the collection and culture of *Amoeba proteus* for class purposes. Proc. Royal Soc. Edinburgh, Vol. 20, pp. 179–182.

TAYLOR, MONICA. 1923. Nuclear divisions in *Amoeba proteus*. Quart. Jour. Micros. Sci., Vol. 67.

TAYLOR, MONICA. 1924. *Amoeba proteus:* Some new observations on its nucleus, life history and culture. Quart. Jour. Micros. Sci., Vol. 69, pp. 119–143. 10 figs.

WAGENER, E. H. 1924. A precipitin test in experimental amoebic dysentery in cats. Univ. Cal. Pub. Zool., Vol. 26, pp. 15–20. 1 pl.

NEW BIOLOGICAL BOOKS

The aim of this department is to give the reader brief indications of the character, the content, and the value of new books in the various fields of biology. In addition there will usually appear in each number one longer critical review of a book of particular significance. Authors and publishers of biological books should bear in mind that THE QUARTERLY REVIEW OF BIOLOGY *can notice in this department only such books as come to the office of the editor. The absence of a book, therefore, from the following and subsequent lists only means that we have not received it. All material for notice in this department should be addressed to Dr. Raymond Pearl, Editor of* THE QUARTERLY REVIEW OF BIOLOGY, *1901 East Madison Street, Baltimore, Maryland, U. S. A.*

CUÉNOT ON ADAPTATION

By John H Gerould, Dartmouth College

The French have been characteristically strong and even brilliant as encyclopedists. The assembling, summarizing and interpreting to the general reader of results of research by specialists in every field of science, though no longer within the power of a single writer, is now accomplished with considerable success by such an organization as that constructed by Dr. Toulouse, Director of the *École des Hautes Études*, who for his *Encyclopédie Scientifique* divides science and its applications to medicine, education, industry, engineering, etc., into about thirty-four departments, each under the direction of a competent leader who selects specialists with breadth of view and power of popular expression to interpret to the public the results of science in their respective fields by means of handbooks of convenient size (usually about 300 or 400 pages).

Professor Maurice Caullery has been happily chosen as Director of the Department of General Biology and has himself contributed to the series a work on "*Le Parasitism et la Symbiose.*" The fifth independent volume in the field of general biology is "*L'Adaptation*" (G. Doin, Paris, 1925, pp. 420) from the pen of Professor L. Cuénot of Nancy, already well known to the scientific public by his "*La Genèse des Espèces Animales*" and other important works.

Cuénot has approached the questions of how and why organisms are fitted to their environment not only from the standpoint and with the training of a critical biologist but also with the liberal spirit of a broad and open-minded philosopher.

In defining the subject, distinction is made between modifications ("*accommodats*") or somations, which an *individual* plant or animal acquires in an environment new to it, corresponding to the changes of acclimatization experienced by a *group* of individuals and their offspring when introduced into a country new to them, and, on the other hand, hereditary, or "statistical," adaptations. "Statistical" is a term applied to adaptations to a given condition or medium, which are numerically frequent, but not necessarily always present, under such conditions. The webbed foot characteristic of aquatic animals, fleshy leaves of plants of desert or seaboard, dwarf plants of mountain tops, are chiefly under hereditary control and statistically numerous, though not universally found, under these given conditions; that is, not all aquatic vertebrates have webbed feet.

Among plants, especially, it is difficult to distinguish by mere inspection between modifications ("*accommodats*"), which disappear when the seed of the somatically modified plant is sown under normal environment, and hereditary or "statistical" variations. Among animals, usually less subject to environmental influences, "sta-

tistical" or hereditary variations are not so often combined with non-hereditary modifications.

It is very unfortunate in the opinion of the reviewer that this excellent book is marred by the constant use of this confusing and inappropriate term "statistical," as applied to hereditary variations and adaptations. The relative abundance, or statistical feature of hereditary adaptations is a minor matter of doubtful significance. It does not even distinguish such adaptations from modifications ("*accommodats*"), for the latter are also facultative and not universal under a given set of circumstances. The term "ethological" which Cuénot suggests as synonym of "statistical" is likewise equivocal and confusing, for modifications or accommodations are also distinctly ethological. It is difficult to find a term characterizing such adaptations as stable and subject chiefly to the control of hereditary factors without using the word "hereditary." "Stable" may be used, if one has occasion to avoid the use of "hereditary," but not "statistical." "Hereditary" is itself not free from objections, but it is clear and sufficiently distinctive.

The author properly emphasizes the fact, which Weismann and other extreme selectionists have passed over lightly, that many organs are nearly or quite useless. As examples, he calls to mind vestigial structures which have ceased to function and also organs which are greatly overgrown (hypertelic) and an encumbrance, such as the splendid plumage of certain male pheasants and birds of Paradise which is so enormous as to make flight impossible, the excessively long horns of the eland, the canines of the sabre-toothed tiger, the grotesquely large thoracic appendages of certain Brazilian leaf hoppers. Moreover certain other organs, internal or external, are useful but not necessary, like the spleen, the uropygeal gland (lacking in certain birds), the air-bladder of fishes the presence or absence of which is evidently a matter of small importance. Cuénot has proved experimentally that amputation of the breathing tube of the water bug, *Nepa*, and the prominent spur upon the femur of the middle pair of legs in the backswimmer, *Notonecta*, the latter supposedly useful for cleaning the mouthparts, interferes in no perceptible way with either insect's activities and functions. The fact that useless or nearly useless organs are sometimes produced leads to an inquiry as to how variations occur, whether at random or in definite directions, fortuitously or orthogenetically.

Cuénot discusses orthogenesis, the type of variation familiar to paleontologists as the tendency of an organ or combination of organs to vary progressively from one geological period to the next, as if tending toward a definite end, e.g., the classic example of the evolution of the hoof from a single toe in the succession of extinct ancestors of the modern horse. Orthogenesis is recognized as a real method of evolution, independent of both natural selection and environmental action. Just as, in the growth of the individual, a mass of unformed tissue shapes itself under unknown internal control into an organ adapted to perform a definite function, so in the history of the race an organ which is at first unformed and useless may acquire a function or may change its function. Orthogenesis signifies then a series of evolutionary changes seemingly directed toward a definite end. In this category the author places the acquirement of a fixed, internally regulated, bodily temperature by the higher vertebrates, the evolution of mammary glands from simple cutaneous glands, the feathers of birds, originating by degrees

from reptilian scales, the electric battery of *Torpedo* transformed from masses of muscle upon the upper jaw, the wing of bat, bird or insect. "It all takes place as if directed toward an end, as if the beginning foreshadowed the conclusion."

Of the three theories which have been advanced to explain the evolution of adaptations: Lamarckianism, Darwinism (or natural selection), and the mutation theory, the last naturally receives the most extensive treatment.

"Darwinism, comprising Lamarckianism by inclusion, is therefore a theory of general application, and its immense success is easily understood; its logic is irresistible: the occurrence of inheritable variations cannot be denied; the tendency toward an indefinite increase in the number of species is certain; and also the destruction of an enormous number of individuals; the conclusion which seems inevitable is the survival of the better adapted, continuous progress, the extension and diversification of life." But while artificial selection involves intelligent choice in the destruction of superfluous individuals, natural selection, in the author's opinion, is nonselective. It involves destruction but not construction.

The discussion of the mutation theory includes a brief critique of recent experiments bearing on the inheritance of acquired characters, all of which are inconclusive. The author denies formally and completely the inheritance of acquired characters, because "it is necessary to have a firm opinion."

Mutations arising in a state of nature vary in natural vigor. They may be (1) lethal and disappear at once or exist only in a latent, compensated state, (2) less viable than the type and gradually disappear (e.g. albinos, mammals with pendant ears), (3) equally viable, and so coexist with the type, thus constituting a polymorphic species, or (4) more vigorous than the type, like the melanic mutation of the peppered moth, tending to supplant.

The occupation of any newly established environment from which former inhabitants, if any, have disappeared is due, Cuénot points out, to the filtering in, from neighboring regions, of species already *preadapted* to the new environment. Thus by trial and error organisms adapt themselves to new regions. To survive, they must already be sufficiently adapted both as to instincts and to structure.

The success of a species arising by mutation in establishing itself depends upon "differential fertility," the two elements of which are the number of eggs laid and the death rate. Cuénot gives a formula for the annual loss of individuals in a race numerically constant, based directly upon the number of eggs produced by a single female and inversely upon the death rate.

To a Darwinian selectionist differential mortality means the survival of the fit and continuous progress by constructive selection; to the mutationist differential mortality means the elimination of the weak and abnormal, the maintenance of a healthy mediocrity; moreover mortality to a large extent is non-selective, the strong and the weak, the adapted and the non-adapted, being indiscriminately eliminated.

The study of orthogenetic series in paleontological succession shows that mutation may result sometimes in organs which are disproportionately large and cumbersome or sometimes completely atrophied. Alternation occurs between periods of mutation and of establishment of mutants as species. Certain points in the organism are more plastic than others, more subject to orthogenetic change. In the main, the changes are small and gradual; others necessarily involve a

profound structural and physiological alteration affecting the whole body, as when a left-handed type of snail give rise to right-handed descendants, no intermediate condition being possible. Rarely does nature make use of large, abrupt variations such as those which under artificial selection have produced, for example, the bull-dog, the ancon sheep, the rumpless fowl. On the contrary variation occurs by "gradual changes affecting in succession different organs, the coördination of which constitutes the adaptation to the environment."

Denying the explanations of adaptive evolutionary change advanced by Lamarck and Darwin, viz.; the inheritance of acquired characters and natural selection, the mutation theory has nothing to offer as a substitute. Cuénot acknowledges the need of discovering some new principle, or factor, internal or external, capable of controlling variations and directing them toward a definite end. This need is especially felt in studying such complex organs as the eye or the ornamentation of organs of such rare and useless beauty as the feathers of the peacock or *Argus* pheasant. Simply stated, we need to know how growth is controlled and differentiation determined.

In Part Three Cuénot interestingly describes many concrete cases of adaptation, the underlying principles of which have already been stated. The conclusion is reached that non-inherited modifications tend to be associated with hereditary variations of similar nature. Thus one may find on a mountain-top plants of a given species which owe their adaptation in various degrees to hereditary and to environmental factors; some are in the condition of non-hereditary modifications and would revert to the lowland type when removed to the plains; others are hereditarily fixed and would not thus respond.

Finally Cuénot discusses the metaphysics of the stupendous problem of adaptation, distinguishing between the mechanical or "efficient" cause, or how adaptations have originated, and their purpose, or why they have come to exist. Teleological concepts can be properly applied only to man's own instruments and machines. It is absurd to assign a final cause (ultimate purpose) to a phenomenon like the rainbow or sunlight clearly independent of mankind. Not less so is the application of such concepts to most organic phenomena.

But organs have useful functions. They are, in a sense, purposeful; and this usefulness of organs, individuals, or species, is only a fragment of a larger usefulness which includes all living things, because, though individuals die, life as a whole flows on. The ultimate biological adaptation is the conservation of life upon the earth. Can we assign to this a purpose?

The three answers that have been given to this question are spiritualism (in a broad sense), mechanism and agnosticism.

"Spiritualism" implies that life has come from the inorganic by an act of divine will. It does not necessarily imply, the author maintains, the doctrine of vitalism, but it usually implies that the universe is biocentric, adapted to production and perpetuation of life, and anthropocentric or adapted to the needs of man. "The details of the world are not made for man, but, if one believes in a spirit as Creator and Organizer of the universe, one may imagine the following sequence as willed by him: the Universe, Life, Conscience." Yet, quoting with approval the idea of Richet: "The expression *to will* is terribly human, but no one can refuse to recognize that gradual development of life and intelligence was included in the destiny of the terrestrial globe." The author thus seems to subscribe to the doctrine of predestination.

Materialistic mechanism on the other hand recognizes no organizing intelligence; its architecture is the result of a fortuitous concourse of atoms. Science discovers only a succession of events in the relation of cause and effect, involving neither volition nor direction; it is occupied with the "how" not the "why" of things, because there is no "why." The monist's universe is without beginning, purpose, or end, but perpetually in a state of evolution. Life is a property of inorganic matter, and under proper physical conditions has developed spontaneously from it either on this or on some other planet. There are in the living organism only physico-chemical properties, though of such complicated nature as to warrant the existence of distinct biological sciences. That some chemist some day may synthesize the living from the non-living is not beyond the bounds of expectation. The apparent purposefulness in organisms, which tends to perpetuate the life of the individual or of the species, is fortuitous unintentional, the result of the elimination, of the unfit, the ill-adapted. As Epicurus long ago held, the bird flies because it has wings, and one may not say that it has wings for the purpose of flight; man sees because he has eyes, the product of a slow evolution of which sight is the result. Man naïvely thinking of the universe in terms of human purpose is merely a transitory excrescence like mould upon the earth's crust, or passes like a flash into oblivion.

Agnosticism takes a middle ground between the two doctrines. For the agnostic the universe is the manifestation of a power which we cannot know and yet which we need to know in order to explain nature. Unable to escape the teleological impression which the universe gives him, the agnostic repudiates more or less clearly the idea of design. He "neither denies nor affirms the existence of a Creator" and "carefully discards all that is metaphysical as unknowable and consequently uninteresting."

Science, occupied as it is solely with secondary causes, is unable to harmonize the conflicting views of those who think in terms of "spiritualism" or of materialistic mechanism. "Their convictions, both equally incapable of demonstration upon a scientific basis are a matter of personal feeling. For the one, the questions of beginning, of design, the 'why,' cannot be suppressed, and demand a reply which is found in the idea of God and in the immense synthesis of Universe, Life, and Man, the grandeur of which must be acknowledged; the second is not disturbed by metaphysical questionings, and the 'how' satisfies his curiosity."

Barring the unfortunate use of the confusing and inappropriate term "statistical" to which the reviewer has called attention, the book is throughout clear and exceedingly interesting. Adaptation is truly "*une effrayante question.*" Successive generations of scholars viewing it each from a different angle have sought and found each their own answer. In the state of knowledge of the nineteenth century the formula of natural selection served well, but this stimulating book shows the inadequacy of this solution and states the problem as geneticists of the twentieth century see it. The present position may be weak and little more than a confession of ignorance. For in spite of all our knowledge of the morphology of chromatin, the touchstone of hereditary characters, we know nothing definite about the determination of form in growing organisms nor about any guidance of variation, but is not a confession of ignorance the sign of an inquiring spirit, and may it not mark progress toward a deeper knowledge of evolution and of adaptation?

EVOLUTION

THE WAYS OF LIFE.
By Richard Swann Lull. *Harper & Brothers*
$3.00 8¼ x 5¾; xiii + 365 *New York*

This is an entertaining popular book on organic evolution with, quite fitly, a good deal of emphasis on the paleontological evidence. It can be highly recommended to the lay reader who wishes to inform himself about the concrete evidence regarding organic evolution.

LIVING ORGANISMS. *An Account of Their Origin and Evolution.*
By Edwin S. Goodrich.
Oxford University Press,
7¾ x 5¼; 200 *New York*

An introduction to organic evolution by the Linacre Professor of Zoology and Comparative Anatomy in the University of Oxford. While containing little that is original, the book is an excellent brief review of the present status of knowledge regarding evolution, written with critical judgment and in an easy readable style. The evidence reviewed is mainly zoological.

THE EVOLUTION OF MAN. *Essays.*
By G. Elliott Smith.
Oxford University Press,
8 s. 6 d. 9 x 5¾; vii + 157 *New York*

A republication of three essays originally prepared for delivery at popular lectures. The first has the title, "The Evolution of Man;" the second, "Primitive Man;" and the third, "The Human Brain." The common purpose of all three of these is to throw light on man's pedigree. While all students will not agree with the author's interpretations, his authoritative position in the field of anthropology and the skill and persuasiveness with which he marshals his arguments, combine to make this a book which every serious student of the subject must read. The zoologist will be especially interested in the material on *Tarsius.*

THE ORIGIN AND EVOLUTION OF THE HUMAN DENTITION.
By William K. Gregory.
The Williams & Wilkins Co.
$6.50 6¾ x 9⅞; xviii + 548 *Baltimore*

This monograph is the standard reference work in the field. It assembles a great mass of evidence as to the fact and method of one phase of human evolution in a clear and convincing manner. A bibliography of 11 pages is appended.

PROMETHEUS OR BIOLOGY AND THE ADVANCEMENT OF MAN.
By H. S. Jennings. *E. P. Dutton & Co.*
$1.00 4½ x 6¾; vii + 86 *New York*

This latest addition to the well-known series of stimulating little books with Greek titles on general aspects of science. In this book the author ascribes greater importance to the environment in determining the nature of living things than most biologists do.

WHAT EVOLUTION IS.
By George Howard Parker.
Harvard University Press,
$1.50 4¾ x 7¼; ix + 173 *Cambridge*

A book of the times, written to meet the demand created by the large and growing popular interest in evolution. The treatment is accurate, clear and concise. It makes its appeal on the clearness and cogency of the reasoning rather than upon illustrations.

THE EARTH BEFORE HISTORY. *Man's Origin and the Origin of Life.* *By Edmond Perrier.* *Alfred A. Knopf* $5.00 6¼ x 9½; xxiv + 345 *New York*

This book, which is one of the volumes in the great series, "The History of Civilization," is, on the whole, the best available account within moderate compass of the evolution of life on the earth, up to man. It is not a popular work in the sense of being easy reading, but it more than compensates in its thoroughness and breadth for any possible lack of popular appeal. It will stand as an authoritative work of the first rank.

GENETICS

GENETICS AND EUGENICS. *A Textbook for Students of Biology and a Reference Book for Animal and Plant Breeders.* *By W. E. Castle* *Harvard University Press* 9¼ x 6½; viii + 434 *Cambridge,*

A new edition of this well-known standard text in genetics. The material has been brought up-to-date. The viewpoint of the book is, of course, Mendelian in an absolutely orthodox sense. The treatment of the biometric side of genetics is not adequate.

MANUAL OF DAIRY CATTLE BREEDING. *By John W. Gowen.* *The Williams & Wilkins Co.* $3.00 6 x 9½; 113 *Baltimore*

This is a laboratory manual to accompany the author's well-known treatise on *Milk Secretion*, published last year. From an intellectual viewpoint it represents a great advance in the laboratory teaching of animal husbandry in our agricultural colleges.

HEREDITY IN NERVOUS AND MENTAL DISEASES. *An Investigation by the Association for Research in Nervous and Mental diseases.* *By various authors.* *Paul B. Hoeber, Inc.* $3.75 9 x 6; xxvii + 332 *New York*

A series of papers by sixteen well-known contributors on various aspects of the inheritance factor in nervous and mental diseases, presented at the meeting of the Association for research in these subjects held in December, 1923. The volume contains little that is really new, but furnishes a useful summary of the present state of opinion in the field covered.

THE INHERITANCE OF MENTAL DISEASES. *By Abraham Myerson.* *The Williams & Wilkins Co.* $5.00 6 x 9; 336 *Baltimore*

This book, though badly written and badly edited in a style which constantly irritates the reader, nevertheless contains a mass of interesting, original observations on the hereditary factor in various abnormal mental conditions. A large number of detailed case histories are given. The work is one which cannot be neglected by any student of human genetics.

GENERAL BIOLOGY

THE SCIENCE OF BIOLOGY. *An Introductory Study.* *By George G. Scott.* *Thomas Y. Crowell Co.* $3.50 8¾ x 5¾; xii + 617 *New York*

This textbook is intended for elementary biology classes in colleges. It is unusually broad in its scope on both the animal and plant sides and appears to have been compiled with excellent judgment.

TEXT BOOK OF BIOLOGY. *For students in general, medical and technical courses.*
By William Martin Smallwood.
Lea and Febiger
$3.75 9¼ x 6¼; xv + 393 *Philadelphia*

A new edition of an old and well established text book of general biology, which has proven its merit in earlier editions.

REGENERATION. *From a physico-chemical viewpoint.*
By Jacques Loeb.
McGraw-Hill Book Co. Inc.
$2.00 9¼ x 6¼; ix + 143 *New York*

In this book, which, to the great loss of biology and the deep sorrow of his many friends, proved to be his last, Doctor Loeb brought together and rounded out his researches on regeneration in *Bryophyllum*. The essential result of the study was to show that equal masses of isolated sister leaves produced under equal conditions of illumination, temperature, etc., approximately equal masses of shoots and roots in equal time. On the basis of this mass relation Loeb attempted, with his customary ingenuity, to explain all the important phenomena of regeneration. Whether this theoretical interpretation is finally accepted or not, it will certainly prove enormously stimulating to research, as has so much of Loeb's other work.

ENZYME INTELLIGENCE AND WHENCE AND WHITHER.
By Nels Quevli. *The Colwell Press, Inc.*
6 x 8½; 578 *Minneapolis*

This curious book is written by a registered pharmacist, who believes that enzymes are the ultimate indestructible and invisible units of life and are conscious and intelligent. He furthermore contends that enzymes produce and maintain all living things we see, and that they come from what he calls "the invisible world of life," to which we return when we die. He has read the accessible book literature of biology very widely and has produced an entertaining volume, but it is much to be feared that Augustus DeMorgan would have classed him as a paradoxer.

EXPERIMENTELLE MORPHOLOGISCHE UNTERSUCHUNGEN. *Lipoid-, Glykogen- und Pigmentstoffwechsel (Handbuch der biologischen Arbeitsmethoden. Lieferung 171).*
By Ernst Leupold. *Urban & Schwarzenberg*
M. 6,30. 7 x 10; 150 pp. *Berlin*

This number of the great Abderhalden hand book deals, in great detail, with the histological, physiological and biochemical technique essential to investigation in certain fields of experimental morphology.

THE ORGANIZATION OF LIFE. *A Revaluation of Evidence Relative to the Primary Factors in the Activity and Evolution of Living Organisms, Including a Factorial Analysis of Human Behavior and Experience. By Seba Eldridge, with an introduction by H. S. Jennings.* *Thomas Y. Crowell Co.*
$4.50 9¼ x 6; xv + 470 *New York*

A much too verbose, and consequently tiresome, theoretical discussion of all the major problems of biology, which seems, with singular pertinacity, to reach a conclusion on about all important topics, such as the inheritance of acquired characters, mechanism and vitalism, etc., which is diametrically opposed to that of most biologists who have thought about the matter. The general tone of Professor Jenning's introduction is very faintly laudatory. The book demonstrates that the author is an enormously industrious person.

IMMUNITY IN NATURAL INFECTIOUS DISEASE.
By F. d'Herelle (Authorized English Edition by George H. Smith.)
The Williams & Wilkins Co.
$5.00 6 x 9; 399 *Baltimore*

In this book the author reviews the existing knowledge regarding the phenomenon of immunity to infectious diseases, from the point of view of his theory of the bacteriophage. The result is to make a highly interesting and valuable contribution to general biology. The subject is developed around the concept of the reactions of living things to stimuli. The book is divided into four parts, of which the first deals with the reactions of living matter in general; the second, with its reactions against inanimate agents; the third, with the reactions against bacteria; and the fourth with the ultraviruses and immunity against them. Though primarily written for the bacteriologist and medical man, the general biologist will find this book extremely interesting and stimulating.

HUMAN BIOLOGY

RICHTLINIEN FÜR KÖRPERMESSUNGEN. *(Und deren statistische Verarbeitung mit besonderer Berücksichtigung von Schülermessungen.)*
By Rudolf Martin. J. F. Lehmanns Verlag
2 Marks 9¼ x 6½; 60 *München*

A very useful and sound treatise on the elementary technique of anthropometry. The material was drawn up particularly for the guidance of those working with school children, but it will be found valuable by a beginner in any field of anthropometry. The biometric methods outlined are only the simplest.

BIOLOGY AND HUMAN LIFE.
By Benjamin C. Gruenberg.
Ginn and Co.
$1.72 8¾ x 5½; xiv + 592 + xi *Boston*

This high school textbook covers an enormous range; in fact, there is no department of biology which is not in some degree touched upon. Naturally, it nowhere goes to very great depths. The reference lists of reading, which follow each of the 51 chapters, are excellent.

TUBERCLE BACILLUS INFECTION AND TUBERCULOSIS IN MAN AND ANIMAL. *Processes of Infection and Resistance; a Biological and Experimental Study.*
By Albert Calmette.
The Williams & Wilkins Co.
$8.00 6½ x 9; 714 *Baltimore*

To the general biologist this book is probably the best source from which to get a general comprehensive review of the present status of knowledge regarding the disease which, taking the world as a whole, kills more people than any other one in the normal, regular course of events. The viewpoint throughout this great monograph is primarily biological rather than narrowly medical. It is a book which should find a place on the shelves of every general biological library.

AN APPROACH TO SOCIAL MEDICINE.
By Francis Lee Dunham.
The Williams & Wilkins Co.
$4.00 6 x 9; 242 *Baltimore*

A sane and somewhat philosophical guide to the social worker and especially to the worker in hospital social service. The student of any aspect of human biology will find it well worth reading.

APHORISMEN ZUM HEILPROBLEM. (*Moderne Biologie Heft 9.*)
By Prof. Dr. Hans Much. *Curt Kabitzsch*
Rm. 2 7½ x 5; 101 *Leipzig*

This is a highly thoretical discussion of the biological nature of disease, its course, and its treatment. A new therapeutic agent, "Omnadin," is discussed and temperature charts showing its action in fevers are given. From the rather vague statements given as to its composition omnadin seems comparable not to a shot-gun prescription, but to a whole flock of shot-guns.

DAS FRAUENPROBLEM IN IDEALSTAAT. *Der Vergangenheit und der Zukunft.*
By Margaret Weinberg. *Curt Kabitzsch*
7¾ x 5; vii + 85 *Leipzig*

An ultrafeministic tract of uplifting tendencies. The most interesting feature of the book is the historical treatment of feminism from the time of Plato down to date. There is a bibliography of three pages and a full index.

BIRTH CONTROL: FACTS AND RESPONSIBILITIES. *A Symposium Dealing with this Important Subject from a Number of Angles.*
Edited by Adolf Meyer. Chapters by Adolf Meyer, Margaret Sanger, Raymond Pearl, E. A. Ross, E. M. East, C. A. Rubenstein, Herbert A. Miller, R. McC. Chapman, R. A. Spaeth, C. C. Little, L. J. Cole, and Eleanor R. Wembridge.
The Williams & Wilkins Co.
$3.00 5¼ x 7½; xiv + 157 *Baltimore*

A treatise, of enormous respectability, on various aspects of birth control. It is made up of a number of separate and quite unconnected essays by different persons, as indicated above. Probably the most significant essay in the volume is the last one, by Doctor Wembridge.

THE HAPPY CHILD.
By Dr. Henry L. K. Shaw (Editorial Adviser) and eight contributors
Dodd, Mead & Co., Inc.
$1.00 5¼ x 7¾; lx + 126 *New York*

This is a book of excellent advice to young mothers, by eight persons of eminent standing in the social and medical fields. The period covered by the advice ranges roughly from birth up to school age.

THE CONQUEST OF DISEASE.
By David Masters.
Dodd, Mead & Co., Inc.
$2.50 5¼ x 7¾; xv + 353 *New York*

This is a treatise, in popular language and style, on the outstanding points in the historical development of preventive medicine and public health. It is much better written than most books of its type, and can be recommended to the lay reader. Secondary school teachers of science will find it very useful.

DIE BIOLOGIE DES KRIEGES. *Die Ueberwindung des Krieges.*
By G. F. Nicolai. *Orell Füssli*
Fr. 25 (Bd. I and II). *Zürich*
I. 6¼ x 9; xx + 324; II. 6¼ x 9; iv + 226

A new edition, with an introduction by Romain Rolland, of a well-known book which got the author into serious trouble when it was first published during the war. It is an interesting and stimulating book which no student of human biology

can afford to neglect, whatever may be his opinion as to the soundness of the conclusions reached.

ESSAYS IN PSYCHOPATHOLOGY. *Nervous and Mental Disease Monograph Series No. 43.*
By William A. White.
$2.50 6¼ x 9¼; x + 140 *New York*

A collection of essays and book reviews by a distinguished psychiatrist. The fundamental viewpoint of the book is that in the first place, man is not separate and distinct from his fellows or from the other components of his environment, but that he is only a place where for the time being certain forces are nucleated, and that as these forces grow out of the environment they resolve again into it; secondly, that so far as man is a container of energy he is not a closed but an open energy system, constantly deriving energy from the outside, constantly giving up energy to the outside; thirdly, that in considering man it may be useful to consider him in terms of energy rather than in terms of organs. These ideas can hardly be regarded as particularly original, but they are developed in an interesting way by the distinguished author.

GEBURTENRÜCKGANG UND RADFAHREN. *Ein Beitrag zum Problem des verschärften Geburtenrückgangs seit 1900.*
By J. Winkelhagen.
Selbstverlag des Verfassers
5¾ x 8¼; 50 *Hamburg*

The secret is out at last! Many people have puzzled their heads over the cause, or causes, of the world-wide decline in the birth rate in recent years. It is all due to riding bicycles, which also has sad effects upon the mental and physical characteristics of the offspring of those who indulge in this dreadful form of locomotion.

ZOOLOGY

ANATOMY AND PHYSIOLOGY OF THE HONEY BEE.
By R. E. Snodgrass.
McGraw-Hill Book Co., Inc.
9½ x 6¼; xv + 327 *New York*

This book is a model of what a biological text ought to be. In a masterly way the author brings together and digests all that is known about the anatomy and the physiology of the honey bee, with the accurate, critical touch of one who knows the subject deeply and widely. It is a great pity that there are not available more treatises of this sort for the common forms of animal life.

FISHES OF THE GULF OF MAINE. *Bulletin of the U. S. Bureau of Fisheries, Vol. XL, Part I.*
By Henry B. Bigelow and William W. Welsh.
Government Printing Office
$2.00 10¾ x 7¾; 567 *Washington*

An excellent account of the taxonomy, with many ecological notes, of the fish fauna of the North Atlantic coastal region. A type of government publication much to be commended.

BRITISH BIRDS (Volume I).
By Archibald Thorburn.
Longmans, Green & Co.
$5.50 6 x 9; xii + 176 *New York*

This is the first volume of a new edition, to be completed in four volumes, of Thorburn's well-known treatise on British birds. The feature of the work, of course, is the beautiful colored plates from the author's masterly paintings. Indeed, the plates may almost be said to constitute the work, for the text is trifling in amount. The original edition was expensive. The present is a popular edition greatly reduced in price. The reproduc-

tion of the plates on the smaller scale has, on the whole, been extremely well done. They make a strong appeal artistically as well as scientifically. This volume deals with the *Passeres* and contains 48 of the 192 colored plates which the whole work will include. It will be a useful addition to the library of any American bird lover.

JUNGLE DAYS.
By William Beebe. *G. P. Putnam Sons*
$3.00 6¼ x 9; v + 201 *New York*

This latest addition to the author's list of popular books on natural history will be found just as fascinating and thrilling as its predecessors. The observations with which it deals center about the jungle laboratory at Kartabo.

A LIST OF BRITISH APHIDES. *Including Notes on Their Synonymy, Their Recorded Distribution and Food Plants in Britain, and a Food-Plant Index.*
By J. Davidson.
Longmans, Green and Co.
$4.50 5¾ x 8¾; xi + 176 *London*

This is a thorough piece of taxonomic work, giving check lists of the aphid species of Great Britain, together with their important synonyms, their food plants, and their distribution. There are also lists of the genera and important synonyms; of the food plants upon which the *Aphides* in the first section have been recorded in Britain, together with the species of *Aphides* found on them; and, finally, a bibliography of 18 pages.

INSECTS AND DISEASE OF MAN.
By Carroll Fox.
P. Blakiston's Son & Co.
$4.00 6¼ x 9¼; xii + 349 *Philadelphia*

This is a systematic textbook of medical entomology for students of medicine and public health. The flavor of the book throughout is taxonomic. It will furnish the student a sound grounding in this field.

BOTANY

BACTERIOLOGY. *A Text Book on Fundamentals.*
By Stanley Thomas.
McGraw-Hill Book Co., Inc.
$2.50 9¼ x 6¼; xiii + 201 *New York*

This is an elementary general textbook on bacteriology for all kinds of undergraduate students, but it is especially directed towards the needs of the engineering student. It is a book which might well be recommended to the lay reader who wishes to gain, in short compass, a sound knowledge of the significance of modern bacteriology. It is clearly written and illustrated. Its greatest defect is that the references to the literature are meager in number and special in character, and, therefore, not well designed to guide the general reader in his progress to further and wider knowledge.

DICTIONARY OF BOTANICAL EQUIVALENTS. *German-English, Dutch-English, Italian-English.*
By Ernst Artschwager; French-English, by Edwina M. Smiley.
The Williams & Wilkins Co.
$3.25 7½ x 5; 103 *Baltimore*

A handy little dictionary of technical botanical terms in the languages specified in the title, which now appears in a second edition.

GENERAL SYSTEMATIC BACTERIOLOGY. *History, Nomenclature, Groups of Bacteria.*
By R. E. Buchanan.
The Williams & Wilkins Co.
$6.00 6 x 9; 400 *Baltimore*

This text-book is the first volume in a projected series of monographs in the

general field of systematic bacteriology. Of the three parts into which the book is divided, the first deals with the classification of the genera and higher groups of bacteria from an historical viewpoint, giving in detail the different schemes of classification which have been proposed in the period 1773 to 1922. The second part deals with biological codes of nomenclature and their application to the specific nomenclatorial problems of bacteriology. Finally, the bulk of the book is made up of an alphabetical list of the names which have been applied to groups of bacteria of higher rank than species—that is, genera and sub-genera—and their present nomenclatural status. The book closes with a bibliography covering 28 pages and indices covering 37 pages. A solid, substantial reference work.

BAKTERIOLYSE. *Einsichten und Aussichten (Mit besonderer Berücksichtigung der d'Herelle- und Much-Lyse). Moderne Biologie Heft 9.*
By Dr. Artur Haim. *Curt Kabitzsch*
Rm. 2 7½ x 5; 84 *Leipzig*

A concise account of the phenomenon of lysis in bacteria which discusses, without reaching any final conclusions, the probable biological nature of the processes involved. There is a bibliography of 13 titles.

PHOTOSYNTHESIS. *The Assimilation of Carbon by Green Plants.*
By Walter Stiles.
Longmans, Green & Co.
$5.50 5¾ x 8¾; vii + 268 *London*

This is a technical work of first rank, assembling and reviewing critically the present state of knowledge regarding photosynthesis in plants. It will naturally and necessarily find a place in every biological library which makes any claim to adequacy.

MORPHOLOGY

A TEXT BOOK OF VERTEBRATE EMBRYOLOGY. *Treated from the comparative standpoint.*
By Ruth L. Phillips. *Lea and Febiger*
$3.50 8 x 5½; xii + 302 *Philadelphia*

A brief elementary text book, well illustrated, and put together with a good deal of pedagogical skill. The first third of the book is devoted to the early development, and the last two-thirds to the embryology of the different organ systems. An excellent summary chapter of 38 pages at the end brings out the significance of the evidence from vertebrate embryology for organic evolution.

THE VERTEBRATE SKELETON FROM THE DEVELOPMENTAL STANDPOINT.
By J. S. Kingsley.
P. Blakiston's Son & Co.
$6.00 6½ x 9½; viii + 337 *Philadelphia*

A ripe and authoritative treatise on comparative vertebrate osteology by a master in this field. There is a bibliography of 21 pages. Excellent indices close the work.

AN INTRODUCTION TO THE FINER ANATOMY OF THE CENTRAL NERVOUS SYSTEM BASED UPON THAT OF THE ALBINO RAT.
By E. Horne Craigie.
P. Blakiston's Son & Co.
$3.00 6 x 8¼; 194 *Philadelphia*

A concise but adequate monograph on the anatomy of the central nervous system of that standard laboratory animal, the

albino rat. It is well illustrated with original drawings and carries a bibliography ten pages in length. Monographs of this type on standard laboratory animals are extremely valuable additions to the literature.

ÉTUDES SUR LES LÉMURIENS. *Anatomie comparée des fosses nasales et de leurs annexes.*
By Max Kollmann and Louis Papin.
Librairie Octave Doin
12 francs 6½ x 10; 61 *Paris*

The fascicle of the *Archives de Morphologie Générale et Expérimentale* is a technical piece of research on the morphology of the nasal fossae in the *Lemuridae*. The most important conclusions reached are: First, that the nasal anatomy indicates that the *Lemuridae* are to be divided into two distinct groups, the Madagascan forms and the extra-Madagascan forms; and, second, that the nasal fossae of *Lemuridae* are constructed on a type absolutely distinct from that of this region in the primates, with the exception of *Tarsius*, which shows the characteristic relations seen in the monkey. The results bring new evidence supporting the conclusion that *Tarsius* is an important form in any consideration of the evolution of the primates.

RECHERCHES ANATOMIQUES SUR L'APPAREIL GÉNITO-URINAIRE MALE DES SIRENIENS.
By G. Petit. *Librairie Octave Doin*
40 francs 6½ x 10; iii + 326 *Paris*

An extensive piece of original research on the male genito-urinary organs of the Sirenians. It constitutes Fascicle 23 of the *Archives de Morphologie Générale et Expérimentale*. There is a bibliography covering six pages.

ALLGEMEINE VERGLEICHENDE FORMENLEHRE DER TIERE.
By Eduard Jacobshagen.
Dr. Werner Klinkhardt
Geh. Mk. 19.
Geb. Mk. 21. 50. 6¾ x 10; viii + 258 *Leipzig*

An excellent treatise on a subject now much neglected in the biological discipline, general comparative morphology. The book is fully and well illustrated and contains a literature list of 202 titles. Translanted into English it would make a first-rate textbook of comparative morphology for American colleges and universities.

PHYSIOLOGY

THE VITAL CAPACITY OF THE LUNGS.
By J. A. Myers.
The Williams & Wilkins Co.
$3.25 6 x 9; 140 *Baltimore*

An interesting, if not very critical, expression of the great revival of interest among clinicians of the measurement of "vital capacity," by which is meant the number of cubic centimeters of air in a single expiration from the lungs. An excellent bibliography, covering 16 pages, is included.

HUMAN PHYSIOLOGY. *A Text-book for High Schools and Colleges.*
By Percy Goldthwait Stiles.
W. B. Saunders Co.
$2.25 8 x 5⅓; 435 *Philadelphia*

The fourth edition of a popular elementary textbook. The book is reliable on technically physiological matters, but not always so on general biological points, as, for example, on page 408 where the statement regarding the hereditary influence of alcohol is directly opposite to the mature conclusions of all the recent critical experimental work in the field.

NUTRITIONAL PHYSIOLOGY.
By Percy Goldthwait Stiles.
W. B. Saunders Co.
$2.25 8 x 5½; 307 *Philadelphia*

The fourth edition of a well-known elementary text on nutrition.

LE MÉTABOLISME DE BASE EN PHYSIOLOGIE ET EN PATHOLOGIE.
By Emile F. Terroine and Edgard Zunz.
Les Presses Universitaires de France
20 francs 6¼ x 9½; 187 *Paris*

A very valuable summary of the present state of knowledge of basal metabolism, developed from the standpoint of physiology by the distinguished Professor of General Physiology in the University of Strasbourg, Doctor Terroine, and from the viewpoint of pathology by Doctor Zunz, the Professor of Therapeutics at Brussels. The book includes a bibliography of 21 pages in length, and is highly to be recommended as an authoritative treatment of the subject. It is published in an interesting new series entitled *Les Problemes Biologiques*, which is a collection of monographs appearing under the patronage of the Technical Committee for Natural Sciences of the *Presses Universitaires de France*.

FOOD AND HEALTH.
By R. H. A. Plimmer and Violet G. Plimmer.
Longmans, Green & Co.
$1.25 5 x 7½; 64 *London*

This is a concise treatise, in popular language and style, setting forth for the lay reader the present state of knowledge regarding the physiology of nutrition. It is well written and illustrated and will form a valuable addition to secondary school and college biological libraries.

HEALTH AND ENVIRONMENT.
By Leonard Hill and Argyll Campbell.
Longmans, Green and Co.
$4.20 8¾ x 5¾; xi + 208 *New York*

In this book is embodied, in rewritten and reëdited form, the substance of three memoirs originally issued as reports of the Medical Research Council, having the titles: "The Science of Ventilation and Open Air Treatment, Parts I and II," and "The Kata-Thermometer in Studies of Body Heat and Efficiency." These reports were of great interest and value to physiologists and it is advantageous to have the material now available in book form for wider circulation. The student of ecology will find this work extremely suggestive.

BIOCHEMISTRY

THE FATS.
By J. B. Leathes and H. S. Raper.
Longmans, Green and Co.
$4.50 8¾ x 5¾; x i + 208 *New York*

A new edition of what is by common consent regarded as *the* authoritative treatise on the biochemistry of the fats. Considerable new material has been added and the extensive bibliography brought up to date.

FUNDAMENTALS OF BIOCHEMISTRY. *In Relation to Human Physiology.*
By T. R. Parsons.
The Williams & Wilkins Co.
$3.00 4¾ x 7¼; xii + 295 *Baltimore*

The second edition of what is one of the best of elementary treatises on biochemistry for the student of general biology. The principal changes from the first edition consist in the addition of a discussion of insulin, and a discussion of Werner's views regarding the constitution of urea. Brief, but well chosen, bibliographies follow each chapter.

PRACTICAL PHYSIOLOGICAL CHEMISTRY.
By Sydney M. Cole.
The Williams & Wilkins Co.
$4.00 9 x 5¾; xvi + 405 *Baltimore*

The sixth edition of a well-known laboratory manual.

THE EFFECTS OF IONS IN COLLOIDAL SYSTEMS.
By Dr. Leonor Michaelis.
The Williams & Wilkins Co.
$2.50 5 x 7¼; 108 *Baltimore*

Contains the substance of lectures given in April and May 1924 by Professor Michaelis at various American universities on the following topics: Adsorption; electric phenomena produced by the adsorption of ions; the origin of the electric double layers; the properties of charcoal; the connection between discharge, adsorption and flocculation; the so-called Donnan equilibrium; lyotropic effects of ions; mixtures of electrolytes. The book lacks an index, which seems a pity, and there are no specific, documented, bibliographical references.

PHYSICAL CHEMISTRY. *Its Bearing on Biology and Medicine.*
By J. C. Philip.
Longmans, Green and Co.
$2.80 7¼ x 5¼; viii + 367 *New York*

In this third edition of a well known elementary text the chief addition has been a discussion of hydrogen ion concentration and buffer mixtures in the last chapter. Throughout the book the material has been brought up to date, with the addition of bibliographical references to recent works. This is one of the best of the numerous books dealing with physical chemistry from the viewpoint of the needs of the biologist.

CHEMICAL DYNAMICS OF LIFE PHAENOMENA.
By Otto Meyerhof.
J. B. Lippincott Co.
8¼ x 5¼; 110 *Philadelphia*

This book is made up of a series of lectures given in the spring of 1913 at various American universities. The topics dealt with are: The physico-chemical mechanism of cell respiration; autoxidations in the cell; chemical relations between respiration and fermentation; the transformation of energy in muscle; the energetics of cell processes. A bibliography of 163 titles is appended.

HANDBUCH DER BIOLOGISCHEN ARBEITSMETHODEN. Lieferung 166. *Fermentforschung.* Containing following articles: *Labfermente*, by S. G. Hedin; *Reindarstellung des Pepsins*, by C. A. Pekelharing; *Arginase und Urease*, by Theodor Sabalitschka; *Alkoholoxydase der Essigbakterien*, by Jakob Meisenheimer; *Darstellung und Nachweis von Oxydasen und Katatasen pflanzlicher und tierischer Herkunft. Methoden ihrer Anwendung*, by Robert Chodat; *Indikatormethoden zum Nachweis von Zelloxydationen*, by Werner Lipschitz; *Reindarstellung der Katalasen*, by H. v. Euler; *Carbohydrasen-Proteasen und Peptasen*, by Julius Wohlgemuth.
Urban & Schwarzenberg
M. 10.50 7 x 10; 260 *Berlin*

The scope of this number of Abderhalden's handbook is sufficiently indicated by the subtitles for the different parts.

THE NATURE OF ENZYME ACTION.
By Sir W. M. Bayliss.
Longmans, Green and Co.
$3.25 6¼ x 9¼; i–viii, 200 *London*

This is the fifth edition of this book, which is published in the series of "Mono-

graphs on Biochemistry." The chief additions and corrections in this edition relate to the methods of purification of enzymes and to the problem of equilibrium in heterogeneous catalysis. The book was put through the press during the lamented author's last illness. There is a bibliography of nearly 20 pages in length.

LA CONCENTRATION EN IONS HYDROGÊNE ET SA MESURE PAR LA MÉTHODE ELECTROMÉTRIQUE. *Application aux variations de l'équilibre acide-base du sang, a l'état physiologique et a l'état pathologique—action de colloides électriques en injection intraveineuse.*

By Maurice Vincent.

Librairie Scientifique J. Hermann

50 cents. 6½ x 10; 102 *Paris*

A useful, concise treatise on the significance of hydrogen ion concentration in various biological matters, but with special reference to the acid-base equilibrium of the blood. There is a bibliography covering 13 pages.

PHYSIOLOGICAL AND CLINICAL CHEMISTRY.

By Wm. A. Pearson and Joseph S. Hepburn.

Lea and Febiger

$4.00 5½ x 8; xvi + 306 *Philadelphia*

A new textbook of physiological chemistry for students of medicine. It is interleaved for notes and is intended to be used both for study and reference and as a laboratory guide. It works out in the end, however, to be essentially an excellent laboratory outline.

BIOMETRY

HANDBUCH DER BIOLOGISCHEN ARBEITSMETHODEN. Lieferung 165. *Wahrscheinlichkeitsrechnung, Methoden der kleinsten Quadrate, Kollektivmasslehre.*

By G. Polya, and *Biometrik und Variationsstatistik, by Paul Riebesell.*

Urban & Schwarzenberg,

M. 6.60 7 x 10; 162 *Berlin*

This number of Abderhalden's Handbook of Biological Technique deals in an elementary but, on the whole, sound way with the theory of probability and biometric technique. For American readers it possesses no advantage over other readily available textbooks in these subjects, but will probably serve a useful purpose in Germany.

AN INTRODUCTION TO THE METHODS OF ECONOMIC STATISTICS.

By William Leonard Crum and Alson Currie Patton. *A. W. Shaw Co.*

$6.00 5¾ x 8½; xii + 493 *Chicago*

Another textbook of statistical methods, in this case with special reference to economic data. It covers about the same ground as other well known textbooks in the field, with rather more attention to the type of correlation which arises in connection with time series than is usually given.

SEX

THE INTERNAL SECRETIONS OF THE SEX GLANDS. *The Problem of the Puberty Gland.*

By Alexander Lipschutz.

The Williams & Wilkins Co.

$6.00 5¾ x 8¼; xviii + 507 *Baltimore*

A general treatise on the endocrine physiology of the sex glands, based primarily on the author's personal researches, but reviewing generally from that point of view the literature in the field. The work is extensively illustrated, and furnishes long bibliographies of the original literature, following each chapter. The sub-

jects treated are: Sexual dimorphism and the secondary sexual characters; the results of castration; the internal secretions of the sexual glands; the seat of production of the internal secretion of the testicle; the seat of production of the internal secretion of the ovary; the sex specific action of the testicular and ovarian hormones; the question as to the isolation of the sexual hormones; the seminal vesicles and the prostate gland; intersexuality; eunuchoidism; sexual hormones and morphogenesis; the problem of rejuvenation. The distinguished authority, Prof. F. H. A. Marshall, says in his introduction that "as a guide to the literature of a branch of endrocrinology that has grown rapidly in recent years the work should be of great value." It is well indexed.

AN INTRODUCTION TO SEXUAL PHYSIOLOGY.
By F. H. A. Marshall.
Longmans, Green & Co.
$2.75 5¼ x 8¼; xii + 167 *London*

This books is intended to meet the needs of biological, medical and agricultural students for a more concise treatment of the subject of sexual physiology than that of the author's well-known book, "The Physiology of Reproduction." It is completely authoritative and admirably suited to fulfil this real need.

SEX AT CHOICE.
By Mrs. Monteith Erskine.
G. P. Putnam's Sons
$2.50 5¼ x 7¾; xxvi + 187 *New York*

This entertaining volume, written in a spirit of deep earnestness and high moral fervor, with a preface by the author's husband, who is a Member of Parliament, sets forth what the author firmly believes to be the true secret of controlling the sex of offspring at will. The theory has but one slight defect; it is not true. The thoughtful reader will find in it an interesting example of the well-known fact that meticulous honesty and sincerity are not the only requirements for a sound and successful scientific methodology.

PUBERTÉ ET MATURITÉ SEXUELLE. (*Étude histologique et expérimentale.*)
By Ed. Retterer. *Librairie Octave Doin*
10 francs 5¾ x 9; x + 208 *Paris*

This is a record published in book form of a series of investigations by the author on the significance of the interstitial cells of the testis and ovary. The general conclusion reached is that these cells play a very slight, if any, rôle in the endocrine activity of these organs.

REJUVENATION BY GRAFTING.
By Serge Voronoff. *Adelphi Co.*
$6.00 5¾ x 8¾; 224 *New York*

This is an English translation of Voronoff's much discussed book, "Quarante-trois greffes du singe a l'homme." The evidence offered falls a long way short of demonstrating that the grafting of anthropoid testicular tissue into men produced any real rejuvenation, or retardation of senescence.

ÜBER DEN INZEST.
By Heinrich Többen. *Franz Deuticke*
3 Marks 10¼ x 7; vi + 92 *Leipzig*

A gruesome record of the case histories of persons convicted of incestuous practices. It has the value of an original document for students of dysgenic and anti-social phenomena.

PSYCHOLOGY AND BEHAVIOR

CHIMPANZEE INTELLIGENCE AND ITS VOCAL EXPRESSIONS.

By R. M. Yerkes and Blanche W. Learned.

The Williams & Wilkins Co.

$3.50 5 x 7½; 157 *Baltimore*

The senior author reviews in the first part of this book rather briefly some of the more outstanding results of his study of the mental traits of a pair of chimpanzees. The second part of the book gives in detail the reduction to musical notation of the various sounds emitted by these chimpanzees in the course of their daily life. A bibliography of 12 titles closes the book, which would have been improved by the addition of an index.

SOCIAL PSYCHOLOGY.

By Knight Dunlap.

The Williams & Wilkins Co.

$4.00 6 x 9; 261 *Baltimore*

A well written treatise by a psychologist of the first rank on the psychological factors or elements in social organizations of various sorts. The topics discussed are: The field of social psychology; sex differences; marriage and the family; religious organization; civic and martial organization; the condition of social progress; the principles of social organizations; and propaganda. The book can be recommended as highly entertaining as well as soundly informative.

ZUM GEGENWÄRTIGEN STAND DER LEHRE VON DEN REFLEXEN.

By Dr. M. Minkowski. *Orell Füssli*

Fr. 4.50 6¾ x 9½ (paper); 61 *Zürich*

This book constitutes the first number of a series of *Neurologische und psychiatrische Abhandlungen* under the general editorship of Professor von Monakow. It is a thorough-going review, with extensive bibliographical documentation, of the present state of knowledge regarding reflexes. A considerable section of the work deals with the interesting subject of the embryonic development of reflex activity.

ALMOST HUMAN.

By Robert M. Yerkes.

The Century Co.

$3.00 5½ x 8¼; xxi + 278 *New York*

This book deals in the main with an account of the well-known colony of anthropoids established and maintained by Señora Rosalia Abreu on her estate near Havana. At the same time it contributes a great deal, out of the author's wide fund of knowledge, to our understanding of the psychology of the primates below man. There is a bibliography of 20 titles, but no index.

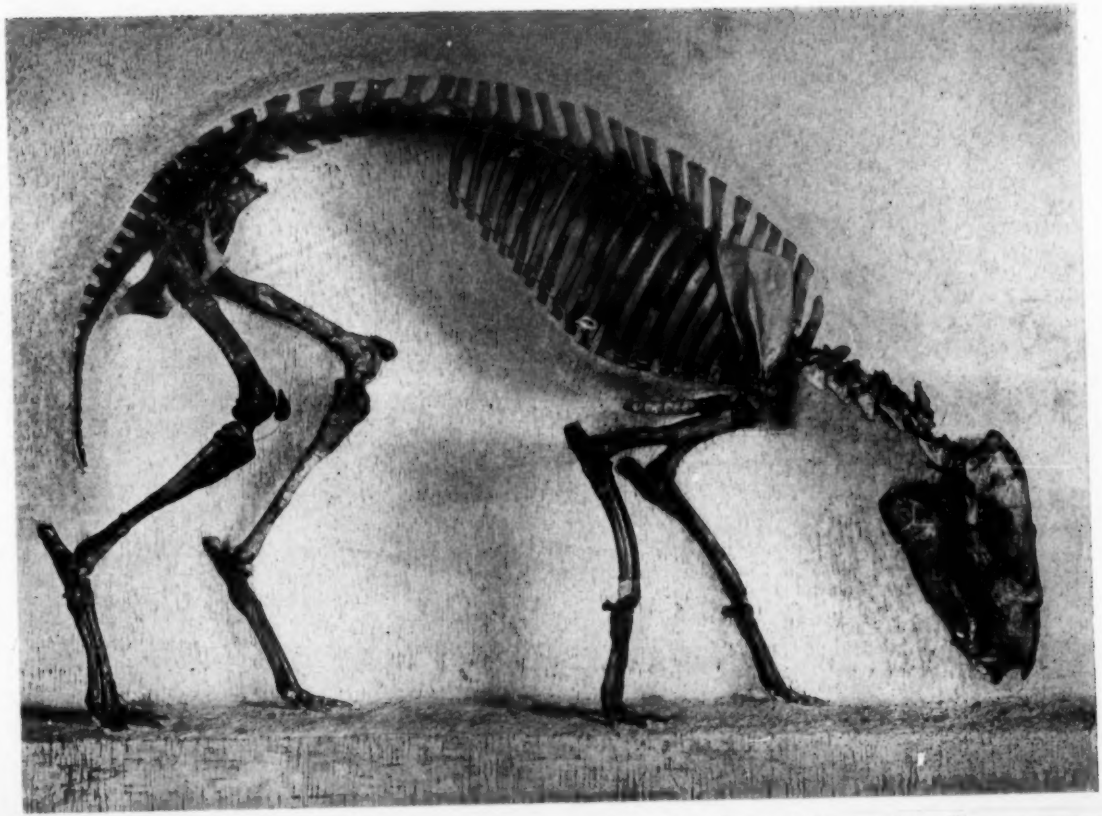
ANIMALS LOOKING INTO THE FUTURE.

By William A. Kepner.

The Macmillan Co.

5½ x 8; ix + 197 *New York*

This a valuable and important little book. It should be in every biological library. It presents in a thoroughly sound and, at the same time, entertaining manner, the established facts regarding the teleological element in the behavior of lower organisms.



Skeleton of Eohippus in American Museum of Natural History, Found in Lower Eocene of Bighorn Basin, Wyoming, in 1911